PEACE/WILLISTON
FISH & WILDLIFE
COMPENSATION
PROGRAM

Mitochondrial and Microsatellite DNA Diversity Throughout the Range of a Cold Adapted Freshwater Salmonid: Arctic Grayling (Thymallus Arcticus)

BChydro

M. D. Stamford
October 2001

BRITISH
COLUMBIA

PWFWCP Report No. 248

The Peace/Williston Fish & Wildlife Compensation Program is a cooperative venture of BC Hydro and the provincial fish and wildlife management agencies, supported by funding from BC Hydro. The Program was established to enhance and protect fish and wildlife resources affected by the construction of the W.A.C. Bennett and Peace Canyon dams on the Peace River, and the subsequent creation of the Williston and Dinosaur Reservoirs.

Peace/Williston Fish and Wildlife Compensation Program, 1011 Fourth Ave.
3rd Floor, Prince George B.C. V2L 3H9

Website: *www.bchydro.bc.ca/environment/initiatives/pwcp/*

This report has been approved by the Peace/Williston Fish and Wildlife Compensation Program Fish Technical Committee.

Citation: M. D. Stamford. October 2001. Mitochondrial and microsatellite DNA diversity throughout the range of a cold adapted freshwater salmonid: Arctic grayling (Thymallus arcticus). Peace/Williston Fish and Wildlife Compensation Program, Report No. 248. 90pp.

Author(s): Mike Stamford[1]
Address(es): [1] Zoology Department, U.B.C., 6270 University Blvd.
Vancouver, BC V6T 1Z4

105-02276/

Mitochondrial and microsatellite DNA diversity throughout the range of a cold adapted freshwater salmonid: phylogeography, local population structure, and conservation genetics of Arctic grayling *(Thymallus arcticus)* in North America

by

Michael D. Stamford
B.Sc. University of B.C., 1996

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE
DEGREE OF MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES
(Department of Zoology)

We accept this thesis as conforming to
the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

© Michael D. Stamford, October 2001

Abstract

The distributions of most Holarctic freshwater fish species were severely altered and restricted during the many glaciation events that have occurred throughout the Pleistocene. Isolation of groups of fish into distinct glacial refugia provided the opportunity for genetic divergence during these periods of allopatry through genetic drift and novel selection pressures. In this thesis, I examined the signature of such isolation and postglacial range expansion in the Arctic grayling *(Thymallus arcticus)* by assaying mitochondrial and microsatellite (nuclear) DNA variation throughout the species' range in North America. I also examined local population structure in the Peace River, British Columbia, because local demographics are integral to a species' phylogeographic structure. I found a dramatic decline in genetic diversity from Alaska to the southeast, which suggests Arctic grayling survived the last ice age in Beringia then bottlenecks and founder events reduced diversity during southward postglacial range expansion. Genetic similarities among regions suggest that Arctic grayling survived glaciation in three refugia north of the ice sheets and in one region south of the ice sheets. A north Beringian lineage dispersed south during the Wisconsinan glaciation and founded populations in an upper Missouri River glacial refuge. These upper Missouri grayling dispersed north postglacially, and founded populations in Saskatchewan and eastern British Columbia. A south Beringian lineage dispersed south from the Yukon River Valley as far as the Peace and Stikine rivers in British Columbia. A third lineage from the Nahanni Valley in the Northwest Territories was more locally distributed in the Mackenzie drainage between Great Slave Lake and the lower Liard River. Population subdivision in the Peace River strongly suggests that Arctic grayling home to their natal stream to spawn. Such local population subdivision and low genetic diversity throughout the species range suggest that Arctic grayling habitat is partitioned among small isolated effective population sizes. Genetic diversity is distributed among lineages on a large geographic scale, and among populations on a local geographic scale. Consequently, to preserve the evolutionary potential of Arctic grayling, several populations within a watershed, several watersheds within a lineage, and several lineages within their geographic range must be prioritized for conservation.

Table Of Contents

Abstract ii

Table Of Contents iii

List of Tables v

List of Figures vi

Acknowledgements viii

Chapter 1: General Introduction 1

- Genetic Consequences of Range Expansion 1
- Divergence in Distinct Refugia 3
- Divergence Among Local Populations 5
- Distribution and Biology of Arctic grayling 5
- Thesis Objectives 7

Chapter 2: Pleistocene evolution and postglacial dispersal of Arctic grayling in North America 9

- Introduction 9
  - *Fossil evidence for glacial refugia* 9
  - *Evidence for two Beringian Refugia* 9
  - *Genealogical Evidence fora Nahanni Glacial Refuge* 11
  - *Contact Zones Among Divergent Lineages* 12
- Materials and Methods 14
  - *Sample Collection* 14
  - *DNA Extraction* 14
  - *Mitochondrial DNA Analysis* 14
  - *Microsatellite DNA* 75
  - *Data Analysis* 16
- Results 18
  - *Mitochondrial DNA Variation Among Lineages* 18
  - *Microsatellite Variation Among Geographic Areas* 20
  - *Geographic pattern of within population diversity* 22
- Discussion 24
  - *Divergence among Arctic grayling mtDNA lineages* 25
  - *Founding and postglacial dispersal of the Nahanni lineage in North America* 26
  - *Founding of Arctic grayling in Beringia* 27
  - *Postglacial dispersal from Beringia and founding of populations in previously glaciated regions* 30
  - *Range expansion from a south Beringian glacial refuge* 31
  - *Implications for Conservation of Arctic grayling* 32

Chapter 3: Local population structure of Arctic grayling in the Peace River, British Columbia **60**

Introduction 60

Site Description 62

Materials and Methods 62

Mitochondrial DNA 63

Microsatellites 63

Data Analysis 63

Results 64

Mitochondrial DNA Diversity 64

Microsatellite Diversity and the distribution of alleles 64

Genetic divergence among Peace River populations 65

Discussion 66

Nuclear DNA insights into postglacial dispersal into Peace River 66

Population structure of Peace River Arctic grayling 67

Conservation Implications of Peace River Arctic grayling 69

Chapter 4: General Discussion **79**

Origin and diversification of Arctic grayling in North America 79

Conservation Genetics of Arctic grayling 80

References **82**

List of Tables

Table 1: Sample sites, sample sizes, haplotype diversity and nucleotide diversity of Arctic grayling mtDNA 35
Table 2: Sample sites, sample sizes and diversity measures (mean # of alleles and heterozygosity) from five microsatellite loci for all Arctic grayling samples. The diversity measures were derived from transformed allele frequency (see text) and sample sites are listed from the northwestern (Beringia at the top) to the southeastern (Saskatchewan) part of the species' range in North America (see Figures) 36
Table 3: List of primer sequences, references and PCR annealing temperatures (hot/cold) used on the five microsatellite loci found variable in Arctic grayling 37
Table 4: List of restriction enzymes, recognition sequence (base pairs) and site matrix for each mtDNA fragment pattern found 38
Table 5: Composite haplotypes of Arctic grayling mitochondrial DNA showing the frequency of each haplotype within sampled populations 39
Table 6: Average percent nucleotide divergence (Nei and Miller 1990) among and within haplotype groups. Range of values are in parenthises (no range for group C as there were only two haplotypes and one divergence measure) 40
Table 7: Analysis of mtDNA variance (AMOVA) among populations grouped into geographic hierarchies on either side of the Rocky Mountain divide (East vs West) and putative origins from glacial refugia (NB = North Beringia, SB = South Beringia, N = Nahanni, NB *(vicar)* = Saskatchewan and Montana populations) 40
Table 8: Analysis of nuclear DNA (microsatellite) variance among populations grouped into geographic hierarchies on either side of the continental divide (East vs West) and putative origins from glacial refugia (NB = north Beringia, SB = south Beringia, N = Nahanni, NB *(vicar)* = Saskatchewan populations) 41
Table 9: Mitochondrial DNA diversity in Peace River Arctic grayling populations 72
Table 10: Genetic diversity at microsatellite loci in Peace River Arctic grayling populations. N and Na are the sample size and number of alleles per population, respectively. He is expected heterozygosity and Ho is observed heterozygosity per population. There were no significant departures from Hardy Weinberg equilibrium (heterozygosity deficits or excesses; Bonferroni adjusted $p<0.05$) in any population. The Parsnip River population includes all four populations from the Table and Anzac rivers (listed below) combined as one panmictic group 72
Table 11: Analysis of molecular variance within and among Peace River Arctic grayling populations. Regional partitioning was determined by presence of geographic barriers (e.g. Peace River Canyon and Nation River Canyon) that isolate populations from each other within the watershed. Nation River was grouped with both upper Peace and lower Peace populations 73
Table 12: Pairwise Fst (θ, Weir and Cockerham 1984) estimates among populations (lower diagonal) and pairwise probabilities for genotypic differentiation (i.e. P(Fst=0), 10,000 Markov chain steps; upper diagonal). Numbers inside parentheses are standard errors and significance was determined using the Bonferroni adjustment 74

List of Figures

Figure 1: Geographic distribution of Arctic grayling sample sites. Shaded area shows the distribution of *T. acticus* in North America and a small portion of Eastern Siberia (Scott and Crossman 1973) 42

Figure 2: Parsimony network showing mutational relationships among the twelve Arctic grayling RFLP haplotypes (numbers inside ovals). The size of the ovals reflects the relative frequency of that haplotype within the data set. Each slash represents a loss or gain of a restriction site 43

Figure 3: Consensus tree from Wagner parsimony analysis of the site matrix generated from twelve mtDNA RFLP Arctic grayling haplotypes. The numbers at the branch forks are the proportion from 1000 bootstrap replicates that the haplotypes at the branch tips occurred among the trees. Only bootstrap values greater than 50% are shown 44

Figure 4: Neighbor-Joining consensus cladogram of pairwise nucleotide divergence estimates (Nei's *d)* among twelve mtDNA RFLP haplotypes of Arctic grayling. Values at the branch forks are the proportion (%) from 1000 bootstrap replicates that the haplotypes at the branch tips occurred among the trees. Only bootstrap values greater than 50% are shown. Branch lengths are not to scale 45

Figure 5: UPGMA consensus cladogram of pairwise nucleotide divergence estimates (Nei's *d*) among twelve RFLP haplotypes of Arctic grayling. Values at the branch forks are the proportion (%) from 1000 bootstrap replicates that the haplotypes at the branch tips occurred among the trees. Only bootstrap values greater than 50% are shown. Branch lengths are not to scale 46

Figure 6: Consensus tree from Wagner parsimony showing the evolutionary relationships among the three haplotype groups (North Beringian, South Beringian and Nahanni) relative to Arctic grayling from the Kamchatkan Peninsula in Siberia, and rooted with a European grayling *(Thymallus thymallus)* haplotype. The haplotypes (numbers correspond with haplotypes in Figure 2) in this tree were determined using the same eight restrictions enzymes used by Redenbach and Taylor (1999). Numbers at the branch fork show the percentage of times out of 1000 bootstrap replicates the haplotypes at the branch tips occurred among the trees 47

Figure 7: Distribution and relative abundance of haplotypes and haplotype groups throughout the range of Arctic grayling in North America. Shaded area shows the current distribution of the species in North America and and includes the north-eastern tip of Siberia 48

Figure 8: Geographic distribution of genetic diversity at microsatellite loci. Both expected heterozygosity and mean number of alleles per locus (in parentheses) are shown. Arrows show inferred postglacial dispersal routes from glacial refugia, labelled with large font (see Figure 7). Shaded area shows the known distribution of the Nahanni mtDNA lineage 49

Figure 9: Change in microsatellite genetic diversity within populations versus their geographic distance from the middle Yukon River, at the confluence with the Koyukuk River (see Figure 1). The three population groups (Nahanni, north Beringia and south Beringia) were designated from the mtDNA lineage they contain (see Figure 7). Regression lines (for illustration) and r values are shown only for north and south Beringian groups 50

Figure 10: Cluster analysis of genetic distance estimates (C-S Chord Distance and Nei's original distance) from microsatellite allele frequencies among populations. The mitochondrial DNA lineage that is known for each population and the geographic location of the populations within each group are also shown. For all of the trees, numbers at the branch forks are the percentage that the populations at the branch tips to the right of the fork occurred among the trees from 100 bootstrap replicates. Branch lengths are not to scale 51

Figure 11: Allele frequencies at microsatellite loci found among populations groups. The populations were grouped according to the predominant mtDNA lineage they contained (see Figure 7). North Beringia (vicariant) group consists of Saskatchewan populations and the North Beringia group 55

Figure 12: UPGMA cladogram of mtDNA nucleotide divergence estimates among regions. Population groups were suggested from clustering of mtDNA divergences. The populations that were analysed with diagnostic enzymes (see text) were included by appointing the most common haplotype from the respective mtDNA lineage (Group A, B or C see Figure 3) for each individual. Nucleotide divergence is the total branch lengths between the tips of the trees; e.g. 2% divergence between lower Liard and upper Liard populations 58

Figure 13: Relationship between microsatellite diversity (mean number of alleles per locus) and two measures of mitochondrial DNA diversity within populations. Standard error bars are shown for mean number of alleles and haplotypes diversity. Only populations where both types of markers were used were included 59

Figure 14: Location of Arctic grayling sample sites in the Peace River watershed in British Columbia. Large arrows along streams indicate the direction of flow. The asterisk marks the location of W.A.C. Bennet Dam at Peace Canyon, which separates the upper and lower Peace rivers 75

Figure 15: Cluster analysis of C-S Chord (Cavalli-sforza and Edwards 1967) distance estimates calculated from microsatellite allele frequency differences among populations in the Peace River, Wollaston Lake and Great Slave Lake. A: UPGMA. B: Neighbor-Joining Method. Numbers at the branch forks are the percentage out of 1000 bootstrap replicates that the populations to the right of the branch forks occurred among the trees 76

Figure 16: Pairwise stream distance (kilometers) versus pairwise Fst (0, Weir and Cockerham 1984) among Peace River populations. A: Isolation by distance among all Peace River populations. B: Isolation by distance among upper Peace River populations, excluding the Nation River. Pairwise comparisons with fry populations (Parsnip River and Finlay River) are accentuated with larger data points and small points are comparisons between adult populations. C: Isolation by distance among all upper Peace populations (i.e. including the Nation River population). See text for details 77

Figure 17: Comparisons between Fst (θ, Weir and Cockerham 1984) estimates among Peace River Arctic grayling populations grouped to show the influence of geographic barriers. The upper Peace River excluding Nation River group and lower Peace River group of populations are not separated by geographic barriers; all Peace River group included populations that are isolated by Peace Canyon and Nation River Canyon; upper Peace River group included the isolated Nation River population. Error bars are 99% bootstraped confidence intervals 78

Acknowledgements

I would like to thank Peace/Williston Fish and Wildlife Compensation Program (B.C. Hydro) for funding my thesis work and thank Ken Ashley for urging me to take on this project. I would like to thank my supervisor Eric Taylor for challenging my intellect and sharing his depth of knowledge of both evolutionary ecology and postglacial fishes. I thank, Don McPhail for enlightening my understanding of biogeography and diversity among fish populations in glaciated regions, Mike Whitlock for helping me to understand the complexities of population genetics, and Eric Parkinson for innovative ideas that fine tuned my sampling strategy. I would also like to thank Brian Blackman and Gordon Haas for all the long general discussions about Arctic grayling biology.

For technical assistance I thank Bob Land for helping to organize some of the details while planning field work, and sharing contacts and familiarity with people living and working around Williston Reservoir. For technical assistance in the lab I thank Derek Louis and Julie Hoffer, and technical assistance in the field I thank Derek Louis, Kim Wasylowich and Tom Gratton. I would also like to thank all the people who took the trouble to collect and send me samples from British Columbia, Saskatchewan, the Yukon, Northwest Teritories and Alaska: Ken Ashley, Dana Atagi, Larry Bartlett, Brad Benter, Steve Biancolin, Brian Blackman, Jeff Burrows, Robert Clark, Paul Davidson, Fred DeCicco, Jim Fish, Fred Gundersen, Gordon Haas, Eric Knudsen, Arne Langston, Jim Larson, George Low, Peggy Merritt, Bill Morris, Sue Pollard, Eric Taylor, Greg Wilson, Randy Zemlac, the guides at Camp Grayling Fly Fishing Lodge, Black Lake Saskatchewan, the guides at Ed and Margy's at Wollaston Lake, Saskatchewan.

I also thank all my fellow grad students, particularly those in the Taylor/McPhail lab for making a fun and productive working environment. Last, but not least I thank my wife, Kate-Louise, for her unselfishness, patience and support while my mind was preoccupied with completing this thesis.

Chapter 1: General Introduction

Genetic Consequences of Range Expansion

Dramatic climate change throughout the Pleistocene Epoch has profoundly shaped biodiversity in north temperate and arctic ecosystems. Survival over the past two and a half million years depended on the ability of species to tolerate climatic oscillations between frigid glaciation and warm interglacial periods that roughly followed a 90 thousand year cycle (Lindsey and McPhail 1986; McPhail and Lindsey 1970; Pielou 1991). Cold-adapted species were distributed among geographically isolated ice-free regions during glaciation, then as the climate warmed many species were able to expand their range into previously glaciated habitats. Studies of phytogeography have revealed that repeated fluctuations in a species' range combined with long periods of isolation are reflected by the low intraspecific genetic divergence found among regions and low genetic diversity in north temperate species (Avise 1987, 1994; Bernatchez and Wilson 1998; Hewitt 2000). The evolutionary processes of divergence through geographic isolation can be calibrated with empirical studies that link molecular genetics with historical geographic events (Taylor et al. 1997; Orr and Smith 1998). Information from these studies can further be used to gain insight into reproductive isolation at a local geographic scale to form a foundation for conserving the evolutionary integrity of species (Avise 1994; Moritz 1994; Bernatchez 1995; Taylor 1999).

Species that were widely distributed during warm interglacial periods often became fragmented by the advancing glaciers, and divergent genotypes evolved in multiple isolated glacial refugia (e.g. Bernatchez and Dodson 1991; Hewitt 1996; Wilson and Hebert 1998; McPhail and Taylor 1999). Range expansion into novel environments during warm interglacials created opportunities for rapid evolutionary divergence among periodically isolated populations (Mayr 1982; McPhail and Carveth 1992), but many of these populations probably went extinct at the onset of the following glacial event. Within each isolated glacial refuge, reshuffling of the gene pool through founder events may have initiated unique evolutionary trajectories and this genetic divergence was compounded by random genetic drift and differential selection pressures (Avise 1987, 1994; Hewitt 1996; Schluter 1996; Orr and Smith 1998). Generally these repeated bottlenecks and founder events during the Pleistocene caused low genetic diversity in north temperate fauna, as measured with neutral DNA markers, compared to fauna that escaped the effects of glaciation (Sage and Wolf 1986; Bernatchez and Dodson 1991; Merila et al. 1996; Green et al. 1996; Wilson and Hebert 1998; Bernatchez and Wilson 1998; Reis et al. 1999; Taylor et. al 1999).

Avise et al. (1987) introduced the idea of phylogeography to describe the principles and processes responsible for geographically localized intraspecific lineages. An approach using molecular genetic techniques to analyze genotypes on a broad geographic scale has effectively bridged the gap between systematics and population genetics by identifying intraspecific phylogenies using mtDNA. Unique evolutionary properties of mitochondrial DNA (mtDNA) that include uniparental (maternal) inheritance, haploidy and a relatively high mutation rate (~10^{-6} mutations per haplotype per generation; Avise 1994) make this marker especially useful for measuring recent intraspecific divergence which adds an historical (systematic) aspect to genetic differentiation among local populations. As the number of studies examining phylogeography increased, it became apparent that phylogenetic gaps existed across the geographic range of a wide variety of species. The distributions of distinct lineages were often associated with zoogeographic provinces (Avise 1994). Further, differences in phylogeographic structure among species were often associated with differences in ecology. For example, low vagility species often have the strongest phylogeographic structure, composed of a mosaic of divergent mtDNA lineages locally distributed and surrounding historical glacial refugia (Avise 1994). Other species that have greater tendencies for dispersal, however, are often composed of a mixture of divergent mtDNA lineages located within the same region or within the same populations (Avise 1994). Phylogeography has clearly demonstrated that geographic isolation and postglacial dispersal have profoundly affected molecular biodiversity and has brought forward intraspecific genetic divergence as a major component of such diversity.

Although there is a general pattern among some north temperate species, where the greatest genetic diversity occurs in regions located nearest to the putative glacial refugia (Sage and Wolff 1986; Merila et al. 1996; Green et al. 1996; Paetkau et al. 1998), there have been relatively few detailed studies of genetic variation across the range of individual fish species. Theoretically, a progressive decline of genetic diversity with geographic distance away from the putative glacial refuge reflects rapid postglacial range expansion and long distance dispersal (Ibrahim et al. 1996; Hewitt 1996). A less dramatic decline of genetic diversity with distance from the historical refuge is predicted when range expansion happens less rapidly and dispersal distance is more localized, or when dispersal is rapid and continued. A genetic signature of rapid dispersal is common in empirical studies of north temperate species as many have specific ecological requirements so their available habitat was rapidly filled behind the receding glaciers (Hewitt 1996). Empirical studies that do not fit the models of rapid range expansion likely reflect fewer constraints to dispersal during the Holocene. For example there was no evidence for bottlenecks found among the noctule bat *(Nyctalus noctula)* perhaps because whole breeding colonies became established in previously glaciated regions. Ecological conditions deteriorated in their historical glacial refuge, but their ability to disperse and establish new colonies elsewhere enabled populations (colonies) to avoid extinction (Petit et al. 1999).

There have been few studies of phylogeography aimed at examining the signature of postglacial range expansion, yet this approach adds rigor to geological and zoogeographical information traditionally used to identify the geographic locations of glacial refugia. The island nature and limited dispersal ability in aquatic habitats make freshwater fish excellent candidates for studying the genetic consequences of range expansion. Freshwater fish had numerous opportunities for postglacial dispersal during glacial recession, as proglacial lakes and altered stream courses bridged headwaters and flooded vast areas of land. These proglacial lakes drained away only a few thousand years after the glaciers began receding, which has since restricted dispersal to within the watersheds. Consequently predictions can be made regarding the number of intraspecific refugial groups from the sizes and shapes of species' ranges (McPhail and Lindsey 1970; Lindsey and McPhail 1986, Dyke and Prest 1987). The phylogeographic structure has been preserved in a number of wide ranging freshwater fish species that have been shown to consist of distinct lineages that evolved in allopatry during the Pleistocene (Bermingham and Avise 1986; Bernatchez and Dodson 1991; Wilson et al. 1996; Wilson and Hebert 1998). Similarly, there may be a strong genetic signature of range expansion in freshwater fish as severe founder events and bottlenecks would have been preserved by subsequent restrictions to gene flow.

Divergence in Distinct Refugia

The inability to travel across land and through high salinity conditions of the oceans has isolated populations and made them particularly vulnerable to changes in ecology caused by global warming. At the end of glaciation events, proglacial lakes and changing stream courses formed behind the retreating glaciers and created temporary dispersal routes for stenohaline (no tolerance for salt water) aquatic fauna into regions that were once covered by glaciers (McPhail and Lindsey 1970). After the glaciers had retreated and watersheds drained along their present courses, groups of once interbreeding fish became geographically isolated from each other. Variation in climatic conditions and diversity of sympatric species assemblages produced different ecological conditions among these geographically isolated groups (Walters 1955; McPhail and Lindsey 1970; Lindsey and McPhail 1986). Furthermore, gradual warming during the Holocene made southern habitats less suitable for cold adapted species so these freshwater fish either adapted, dispersed northward, or went extinct. There are many examples of natural populations of north temperate fishes where reproductive isolation and novel ecological conditions (conditions required for rapid evolution, McPhail and Carveth 1992) resulted in the evolution of species pairs or more subtle morphological differences (Haas 1991; Thompson et al. 1997; Bernatchez and Dodson 1990; Bernatchez et al. 1996; McPhail and Taylor 1999; Taylor 1999). It is worth noting that species pairs rarely occur in southern regions that escaped the direct effects of glaciation, yet they are common in postglacial lakes and are likely the result of ecological speciation (Schluter 1996; Bernatchez et al. 1999; Turgeon et al. 1999). Both survival in allopatry during the Pleistocene and current ecological conditions in previously glaciated regions have combined to generate biodiversity in north temperate freshwater fishes.

Comparative phylogeography of Palearctic and Nearctic fishes has revealed a clear and predictable distinction between species located in previously glaciated northern regions and unglaciated regions further south (Bernatchez and Wilson 1998). The phylogeographic structure of northern species reflects recovery from recent extinctions that were caused by Pleistocene glaciations. Relatively low nucleotide diversity in northern regions has resulted from displacement by glaciers, lengthy isolation in glacial refugia and sequential bottlenecks during postglacial dispersal. In southern unglaciated regions, greater genetic diversity and divergence among sister species and intraspecific lineages reflects historically large population sizes that remained relatively undisturbed during the Pleistocene glaciations. These differences in genetic diversity and divergence were found among both intraspecific and interspecific groups and, interestingly, changes were located at the same latitude (46° North) as the maximum southern extent of the Wisconsinan glaciation (Bernatchez and Wilson 1998).

Within glaciated regions of North America the Mississippi Refuge had a strong influence on the biodiversity of freshwater fish (Bernatchez and Wilson 1998). This was probably due to extensive formation of glacial lakes in the region so there were ample opportunities for postglacial range expansion into previously glaciated regions (Lindsey and McPhail 1986; Crossman and McAllister 1986; Dyke and Prest 1987; Bernatchez and Wilson 1998). Studies of phylogeography of wide spread cold adapted species like lake trout *(Salvelinus namaycush* Wilson and Hebert 1998) and lake whitefish *(Coregonus clupaeformis* Bernatchez and Dodson 1991) revealed large portions of their range were colonized by lineages that originated in southern refugia (e.g. Mississippi, Missouri and Great Plains). The phylogeographic influence from Arctic refugia was substantially less even though there were vast areas that remained ice free (e.g. Beringia) throughout the Pleistocene that periodically enabled freshwater fish to disperse between Siberia and North America (McPhail and Lindsey 1970; Bernatchez et al. 1991; Bernatchez and Wilson 1998). A plausible explanation for this stems from geological evidence that barriers (persistent Cordilleran ice and waterfalls near the Mackenzie River) existed during the early Holocene that limited range expansion from Beringia (Bodaly and Lindsey 1977; Lindsey and McPhail 1986).

The phylogeography of arctic char *(Salvelinus alpinus)* in North America has confirmed this cold adapted species survived glaciation in eastern (Atlantic) and western (Beringia) arctic refugia (Wilson et al. 1996). Their geographic range however, does not go beyond a few kilometers upstream in coastal arctic watersheds, including those drainages in Alaska and the Yukon Territories that remained ice free for multiple glaciations (McPhail and Lindsey 1970). An isolated population in eastern North America (Maine) and their distribution in Europe, which includes relict populations in southern high elevation areas, suggest they expanded their range inland behind the receding glaciers (Scott and Crossman 1973; Brunner et al. 1998). This apparently limited inland dispersal by North American arctic char from the arctic coast may be due to ecological constraints that cause low survival in landlocked freshwater environments (i.e. they have a strong dependence on anadromous life history). Alternatively, ecological conditions behind the retreating glaciers were beneficial for range expansion by cold adapted species like arctic char and perhaps it was barriers that restricted dispersal inland from Arctic refugia in North America. Global warming during the Holocene may have caused recent range contraction, which isolated southern relict populations in cold high elevation locations in Europe and eastern North America (Scott and Crossman 1973, Brunner et al. 1998). Alternatively, these southern relict populations were founded earlier during the Pleistocene and became isolated by advancing Wisconsinan glaciers and range expansion during the Holocene was more restricted than earlier interglacial periods.

Further studies of phylogeography aimed at examining cold adapted freshwater fish in North America are needed to further define the influence from Arctic refugia. There are a number of widely distributed freshwater fish species (e.g. *Prosopium cylindracaeum, Coregonus nasus, Stenodus leucicthys* and *Thymallus arcticus)* that clearly survived glaciation in Beringia yet their phylogeographic structure has not been analysed with genetic markers (McPhail and Lindsey 1970; Scott and Crossman 1973). Genetic analysis is needed to determine if these wide ranging species survived in multiple glacial refugia as did lake trout and lake whitefish. It needs to be established with greater rigor that barriers restricted postglacial range expansion from Beringia. Perhaps such barriers were not so formidable for some freshwater fish species such that genetic diversity across their range has been heavily influenced by range expansion from Arctic refugia.

Divergence Among Local Populations

Evolutionary divergence within a geographic region may result from aspects of a species' ecology and life history that isolate groups from each other. Geographic isolation allows local adaptation which promotes genetic differentiation among distinct populations found within a geographic region. Genetic divergence at neutral loci among these potentially locally adapted populations may be subtle and difficult to measure because natural selection may act more rapidly than divergence resulting from genetic drift especially at high effective population sizes (e.g. Taylor et al. 2000). Consequently, regions that were recently colonized postglacially may contain populations that are distinct for quantitative traits (the part of the genome that influences fitness), but that might be similar using neutral molecular genetic markers (e.g. Ryman et al. 1979). Furthermore, these locally adapted traits may be maintained even in the face of gene flow among distinct groups, which could homogenize neutral DNA (Taylor 1999). Consequently, genetic divergence measured with neutral genetic markers may be a conservative measure of population distinction (Lynch 1996; Taylor 1999) and there are examples in nature where populations show profound differences in life history and morphology but are very similar at neutral loci (Ryman et al. 1979; Taylor 1999; Taylor and McPhail 1999; Taylor et al. 2000).

Among salmonids, reproductive isolation is most notably due to homing behaviour (spawning site fidelity to natal streams) with limited straying. Spawning site fidelity results in assortative mating which enables genetic divergence and adaptive radiation among spawning populations within a region. Straying between these spawning populations disrupts the isolating effects of homing and tends to homogenize the genotypes of populations that exchange migrants. Straying is an integral part of postglacial range expansion, however, so widely distributed refugial groups might have life histories that include large amounts of straying among local populations. Lineages that originated from ice free regions that were severely impacted by Pleistocene glaciation (e.g. fluctuating extent of ice sheets throughout the Pleistocene likely affected southern refugia to a greater extent than in Beringia) might have wider distributions than lineages that survived in more stable glacial refugia. The wide distribution of Mississippi-origin lineages and species has generally been attributed to greater dispersal opportunities (McPhail and Lindsey 1970, Crossman and McAllister 1986), but some lineages that survived here might also have inherited a tendency to disperse from their pre-Wisconsinan ancestors. Consequently, cold-adapted freshwater fish species (most notably salmonids), that survived in Beringia as well as southern refugia, might display differential homing behaviour among lineages. Clearly investigation of these possibilities requires extensive data collection throughout the range of multiple cold adpated species. Nonetheless, it signifies the potential for differential local population structure among lineages due to different selection pressures for homing among the glacial refugia.

Distribution and Biology of Arctic grayling

Arctic grayling in North America warrant further study of their phylogeography because there is uncertainty regarding the number of distinct lineages that exist throughout their range (Walters 1955, McPhail and Lindsey 1970, Lynche and Vyse 1979, Redenbach and Taylor 1999). The species is continuously distributed in mainland Arctic drainages from the west coast of Hudson Bay to the west coast of Alaska and northern British Columbia, with two southern relict populations in the upper Missouri River and Lake

Michigan (Scott and Crossman 1973). These southern isolated groups may have been founded during postglacial range expansion then became isolated later as their ranges contracted later in the Holocene. Alternatively, vicariant groups were founded during a previous interglacial and then became isolated by expanding Wisconsinan glaciers. The Michigan grayling (extinct since the 1930's) may have persisted in the Mississippi glacial refuge, which raises the possibility that they survived there during the last glaciation (McPhail and Lindsey 1970). Arctic grayling in Montana (upper Missouri River) are genetically distinct from grayling in the northern part of the species' range (Lynche and Vyse 1979; Redenbach and Taylor 1999), which also suggests that they are a vicariant group that survived in a Great Plains, upper Missouri or Mississippi glacial refuge. Morphological evidence suggests however, that there are at least two distinct lineages in the north (McCart and Pepper 1970; Reed 1973), and there is geological evidence that an inland glacial refuge (Nahanni Refuge) harboured freshwater fish fauna in the Northwest Territories (Lindsey et al. 1981; Foote et al. 1992). There are, therefore, potentially five lineages of *T. arcticus* originating from five geographically isolated glacial refugia in North America, yet there is currently only enough genetic evidence to confirm the existence of two (Redenbach and Taylor 1999).

Arctic grayling life history includes a complex migration among early spring spawning habitat, summer feeding habitat, and overwintering habitat (Northcote 1995, Tack 1980). These migrations likely evolved from seeking out pockets of suitable habitats that may be localized and rare (especially overwintering areas) in the climatic extremes of their high latitude or high elevation environments. They reach sexual maturity at age three or four, and they spawn every year until they die between age eight and seventeen (Northcote 1995). Overwintering areas for all life history stages include lakes, deep mainstem rivers and warm spring fed creeks that maintain sufficient flows throughout the winter. In the short growing season of the extreme Arctic, the smallest young of the year may burrow deep into the gravel to survive winter in subsurface flow of streams (J.D. McPhail pers, com.; McCart et al. 1972; Skopets 1991). Little is known about fish movements under the ice, but the rarity of overwintering areas may require that more than one life history stage, and more than one spawning population, spend the winter together at one site. The diversity of migratory cycles, however, observed among Arctic grayling populations throughout their range suggests that migratory cycles are adaptive, and that the local population structure of Arctic grayling most likely involves some degree of homing to their natal stream to spawn (Northcote 1995, 1997).

Tagging studies have found that grayling return annually to the same overwintering and feeding locations that often range over hundreds of kilometers, which suggests they also return to the same spawning locations (Craig and Poulin 1975; Tack 1980; Armstrong 1986; West et al. 1992; Northcote 1995; Blackman 1998). A tagging study of the European grayling *(Thymallus thymallus)* in Lake Mjosa, Norway found that 84.5% of the tagged adults returned to the same stream to spawn annually (Kristiansen and Døving 1996). Although these tagging studies show spawning site fidelity, they do not prove these spawning sites are also the natal streams. Evidence for divergent rheotactic behaviour among inlet and outlet spawning Arctic grayling fry from the same lake in Montana, however, suggests that local adaptation has evolved and been promoted by homing (Kaya 1989; Kaya and Jeanes 1995). There has only been one genetic test for population subdivision of Arctic grayling, but it was unable to distinguish among spawning populations from the same watershed using allozymes (Hop 1985;

Hop and Gharrett 1989). A series of other studies found clear life history and phenotypic differences among these same spawning populations (Armstrong 1986; Hop 1985; Northcote 1995) which suggests that genetic distinctions could also be revealed with molecular markers that have greater variation than allozymes (e.g. microsatellites). Population divergence measured with neutral molecular markers is useful for estimating the extent to which Arctic grayling home to their natal stream to spawn as demonstrated in other salmonids (Angers et al. 1995; Wood and Foote 1996; Wenburg et al. 1998). Strong homing behaviour implies less genetic exchange among populations which, in turn, results in greater genetic divergence and the potential for local adaptation within a watershed.

Clearly, the resolution of genetic structure both at wide and local geographic scales is required in order to understand the evolutionary potential of a species. Distinct lineages distributed within a north temperate species' range evolve through geographic isolation in distinct glacial refugia and, depending on the length of time in allopatry, these lineages may become reproductively isolated when in sympatry (Taylor 1999). Local population structure might be affected by more than one of these lineages (e.g. Bernatchez et al. 1996, Taylor and Bentzen 1993, Thompson et. al 1997), but the evolution of these lineages could also be affected by the local population structure within their ancestral group (Hewitt 1996). Certain local adaptations or geographic locations of distinct populations within the ancestral group may have determined differential survival and dispersal ability during climatic oscillations of the Pleistocene.

The genetic structure of Arctic grayling in North America on both of these geographic scales is largely unknown and is the subject of this thesis. In addition, grayling are highly sensitive to changes to ecology that are often caused by human development (Walters 1955, Scott and Crossman 1973, Northcote 1995). Already one relict population in Michigan has gone extinct and the remaining one in Montana has declined severely, both caused by human impacts to their environment. The species in North America has clearly had a Beringian origin (Redenbach and Taylor 1999), but their widely disjunct distribution (combined with morphological and genetic evidence mentioned above) strongly suggests that other divergent lineages have evolved in multiple glacial refugia (McPhail and Lindsey 1970; McCart and Pepper 1970; Lynche and Vyse 1979; Redenbach and Taylor 1999). If we hope to preserve the evolutionary legacy of this species it is imperative to understand how its genetic diversity is partitioned geographically and how populations interact on a local geographic scale. Such knowledge will facilitate further studies that focus on unique adaptive differences that are not only interesting biologically, but will raise interest and awareness of this unique salmonid. My thesis, therefore, involves the initial steps towards understanding the evolutionary potential of this species, information that is fundamental for conservation.

Thesis Objectives

The purpose of my thesis was to investigate the genetic structure of Arctic grayling from an historical evolutionary and ecological perspective. I used a phylogeographic approach to determine the distribution of distinct refugial groups in North America using two sets of independent neutral molecular genetic markers, mitochondrial DNA and microsatellites. I then used these same markers to investigate how Arctic grayling genetic variation is partitioned on a local geographic scale and how barriers have influenced their dispersal. My thesis is structured into three subsequent chapters. In chapter 2, I describe the phylogeographic structure of Arctic grayling in North America and present molecular genetic evidence for three distinct lineages. The genetic signature of postglacial range expansion revealed that these three lineages actually originated from four Wisconsinan glacial refugia. In chapter 3, I describe the population structure of Arctic grayling on a local geographic scale in the Peace River watershed in British Columbia. Populations in part of the Peace River have declined recently owing to overfishing and habitat loss caused by flooding that resulted from construction of the W.A.C. Bennet dam in 1968. Other populations that exist below the dam have remained relatively undisturbed. The consequences of this recent disturbance and the manner in which Arctic grayling genetic variation is partitioned across the landscape were investigated. In chapter 4, I provide general conclusions and interpretations from the findings of chapters 2 and 3 and discuss how this information can aid in the conservation of Arctic grayling in general.

Chapter 2: Pleistocene evolution and postglacial dispersal of Arctic grayling in North America.

Introduction

Fossil evidence for glacial refugia

The wide distribution of Arctic grayling suggests that they colonized North America postglacially from ice free regions that were located both north and south of the Wisconsinan ice sheets (McPhail and Lindsay 1970). Fossils discovered in the south have confirmed that *T. arcticus was* present in Indiana about 200,000 years ago (Miller et al. 1993) and in southern Alberta between 20,000 and 30,000 years ago (Burns 1991). in the north, fossils found in the Yukon River in Alaska and in the Yukon Territories have confirmed that Arctic grayling were present in early Wisconsinan glacial lakes (McAllister and Harrington 1969, Cuumba et al. 1981). The early Indiana fossil dates back to the pre-Illinoian interglacial, indicating that Arctic grayling were once distributed further south than they are currently. The Illinoian glaciation would have fragmented this wide distribution and populations may or may not have survived in this region; presumably their range expanded again during the Sangamon interglacial (120,000 years ago). Arctic grayling were present in Beringia (Old Crow, Yukon) during this time and then must have dispersed into southern Alberta some time during the Wisconsinan glaciation or just before. The southern Alberta fossil dates to a time when there may have been a brief (4000 years) warming that could have allowed dispersal between the Cordilleran and Laurentian glaciers between 34,000 and 30,000 years ago (Lindsey and McPhail 1986).

Consequently, this is strong evidence that Arctic grayling survived the Wisconsinan glaciation in Beringia, and that they were present in more southern areas before the Wisconsinan glaciation began some 75,000 years ago. The fossil in southern Alberta and genetic evidence (Redenbach and Taylor 1999) suggest that they reached their current wide distribution in North America through postglacial dispersal from northern (Beringia) and southern (Mississippi/Missouri) glacial refugia. Nonetheless, further evidence is needed to confirm that Arctic grayling survived in one or more ice-free region south of Beringia during the Wisconsinan. Furthermore, if there were multiple glacial refugia used by Arctic grayling, what was their influence on current populations? In this chapter I will test the hypothesis that Arctic grayling reached their current distribution by dispersing from glacial refugia located both north (Beringia) and south (Mississippi or Missouri refugia) of the Wisconsinan ice sheets.

Evidence for two Beringian Refugia

Two morphological phenotypes of Arctic grayling located in two distinct geographic regions were discovered in the northwestern part of the species' range (McCart and Pepper 1970, Reed 1973, Bodaly and Lindsey 1977). A large-scale form (low counts along their lateral line) was concentrated in northern Alaska, along the Beaufort Sea and Bering Sea coasts, as far south as Bristol Bay and as far east as the lower Mackenzie River. A small-scale form was concentrated in interior Alaska along the south slope of the Brooks Range and the Yukon River, and ranged as far south as the upper Peace and the upper Stikine rivers in British Columbia. Although morphometric characters are often phenotypically plastic (Dillinger et al. 1991, Lindsey 1981), the stepwise distribution of these two phenotypes on either side of the Brooks Range suggests that they evolved in two isolated regions of Beringia (Crossman and McAllister 1986, Lindsey and McPhail 1986). The large-scale form must have originated in the north slope of Beringia and survived in isolation from the small-scale form that survived in southward flowing watersheds of Beringia. Further evidence for these two isolated regions of Beringia was provided by similarly distributed distinct phenotypes of *Coregonus clupeaformis* and *Salvelinus alpinus* in northwestern Canada and Alaska (Lindsey and McPhail 1986). Isolation and adaptation to Arctic coastal conditions for thousands of generations may have promoted the evolution of an apparently unique euryhaline large scale form of Arctic grayling that is distributed along the Beaufort Sea coast (Walters 1955, Tack 1980).

Close genetic relationships among putative small scaled forms of Arctic grayling in the middle Yukon River, the upper Liard River and the Peace River in British Columbia (Redenbach and Taylor 1999) has revealed a southeast Beringian lineage that probably dispersed into the northwestern part of the species' range in North America. Further genetic evidence from two independent studies found that Arctic grayling in Montana were divergent from populations in British Columbia and Alaska (Redenbach and Taylor 1999) and in tributaries of the Mackenzie River in the Northwest Territories (Lynch and Vyse 1978). These studies, however, could not refute the hypothesis that Montana grayling were founded from a southern Mississippi/Missouri refuge (Lindsey and McPhail 1970) because sample sites were few and concentrated in northwestern parts of the species' range.

In general, south Beringian origin freshwater fish did not expand their range very far into the rest of North America (McPhail and Lindsey 1970; Bernatchez and Wilson 1998). Drainage connections between the Yukon River and the upper Liard River (Mackenzie drainage) were covered in ice, and a barrier on the Mackenzie River impeded early Holocene range expansion from Beringia until well after other opportunities for dispersal into the southeast were eliminated (McPhail and Lindsey 1970, Scott and Crossman 1973, Bodaly and Lindsey 1977). Opportunities for range expansion, however, may have been greater from the Arctic coast of Beringia during the first part of the last glaciation. Climatic fluctuations that occurred during the first 20,000 or 30,000 years of the Wisconsinan glaciation suggest that an ice-free corridor opened periodically between the Cordilleran and Laurentide ice sheets, and extended from the Arctic coast to the Missouri River headwaters (Lindsey and McPhail 1986; Pielou 1991). Freshwater fish fauna living along the Arctic coast of Beringia (e.g. large scaled Arctic grayling) most likely colonized the rivers draining the north slope of this region (located near the lower Mackenzie River) and may have taken advantage of early dispersal routes into the south. Unfortunately the final onset of glaciation, which began between 25,000 to 15,000 years ago, obliterated fossils and other geological evidence in the corridor (Lindsey and McPhail 1986; Dyke and Prest 1987; Pielou 1991). Consequently it is unknown which species, if any, were present in the watersheds that drained the receding glaciers in the corridor.

Nevertheless, a test for genetic relatedness and the genetic signature of range expansion among populations of freshwater fish species that are distributed north and south of the Wisconsinan ice sheets (e.g. Arctic grayling and lake trout) could provide evidence that this ice free region served as a dispersal corridor for freshwater fish. A trend of close genetic relatedness among populations from the upper Missouri River and Arctic regions of Beringia, with no evidence for range expansion between the regions, suggests dispersal took place but advancing glaciers drove populations between the groups extinct (i.e. a "vicariance" scenario). The phylogeographic structure of lake trout *(Salvelinus namaycush)* found such a trend (Wilson and Hebert 1998), however mixing among refugial groups has obscured the signature of range expansion (Bernatchez and Wilson 1998). Arctic grayling might yield more robust evidence for vicariance as the presence of relict southern populations suggests that they were less effective than lake trout at expanding their range. Consequently, I will test the hypothesis that southward early Wisconsinan dispersal was possible for freshwater fish between the Laurentide and Cordilleran ice sheet. I will gather phylogeographic evidence to determine if Arctic grayling survived in two isolated regions in Beringia (which gave rise to the large and small scale phenotypes), and that early Wisconsinan dispersal took place southward from the Arctic coast of Beringia (Beaufort Sea Coast) to the upper Missouri River in Montana.

Low genetic diversity found in the divergent Montana Arctic grayling populations (Lynch and Vyse 1978; Redenbach and Taylor 1999) suggests that this region was founded through postglacial range expansion from a distant glacial refuge (Ibrahim et al.1996; Hewitt 1996). Furthermore, two divergent Montana haplotypes were found (although they were rare) in northern drainages, one in the Peace River in British Columbia and the other in the Yukon River in Alaska (Redenbach and Taylor 1999), suggesting that the upper Missouri River (Montana) was founded by grayling from a northern refuge. Although Redenbach and Taylor (1999) concluded that the upper Missouri was colonized by Arctic grayling from a southern glacial refuge, they had no genetic information from the Arctic coast of Beringia. Consequently, founding individuals could just as easily have arrived in Montana by dispersing southward from Arctic Beringia, losing diversity through founder events and drift along the way (McPhail and Lindsey 1970, Lindsey and McPhail 1986, Hewitt 1996, Ibrahim 1996). Clearly, there is not enough information to distinguish between these alternative hypothetical origins for the divergent haplotypes found in the upper Missouri River.

Genealogical Evidence for a Nahanni Glacial Refuge

The Nahanni Refuge consisted of a series of periglacial lakes in the Nahanni Valley, N.W.T., at the southern tip of an ice-free region that extended southeast from Beringia between the Cordilleran and Laurentide ice sheets. These isolated watersheds existed both during the Wisconsinan and Illinoian glaciation (Ford 1976, Lindsey and McPhail 1986). Freshwater fish that survived there would have had access to early Holocene dispersal routes across barriers and headwaters into the upper Liard, upper Peace and Fraser systems (Lindsey and McPhail 1986). Given this evidence for an important source of diversity in glaciated North America, there is surprisingly little rigorous phylogeographic evidence that freshwater fish survived glaciation in the Nahanni refuge.

The phylogeography of lake whitefish *(Coregonus clupeaformis,* Foote et al. 1992) and lake trout *(Salvelinus namaycush,* Wilson and Hebert 1998), both widely distributed cold adapted freshwater fish species, have revealed distinct lineages that are associated with an interior glacial refuge. Foote et al. (1992) found unique allozyme alleles that were strongly associated geographically with the Nahanni Valley, but Bernatchez and Dodson (1991) found no evidence for a Nahanni lake whitefish refugial group using mtDNA. The Nahanni lake whitefish populations that were identified by Foote et al. (1992) were not included in Bernatchez and Dodson's (1991) work so the phylogeny that was inferred from allozyme data has never been rigorously validated with mtDNA analysis. A 'Nahanni' lineage is also uncertain for lake trout because Wilson and Hebert (1998) never analyzed samples located near the Nahanni Valley. Furthermore, the distribution of the putative Nahanni lake trout lineage could also be explained by range expansion from south Beringia (Yukon River valley) because the same distinct haplotypes were distributed in the Yukon River and coastal drainages of Alaska and the Yukon Territories. Clearly, although existing evidence for a Nahanni glacial refuge is intriguing, it needs to be validated with further study.

Judging from their wide distribution in North America, Arctic grayling might also have survived glaciation in the Nahanni refuge and expanded their range postglacially beyond barriers into the upper Peace and upper Liard rivers. They might also have had access to postglacial dispersal routes leading to the upper Missouri River and as far east as the Great Lakes (Lindsey and McPhail 1986). Clearly, the phylogeography of Arctic grayling in North America can not be fully understood without analyzing populations located near putative glacial refugia, including the Nahanni Valley. I will test a third hypothesis in this chapter: that Arctic grayling survived the Wisconsinan glaciation in the Nahanni Valley. Evidence for such a scenario would be a distinct lineage of Arctic grayling mtDNA located in and around the Nahanni Valley. Given the close proximity of the Nahanni Valley to postglacial dispersal routes, it is likely that the divergent haplotypes found in the upper Missouri River originated from this putative Nahanni Refuge. Such widespread postglacial range expansion in North America would result in greater genetic diversity near the Nahanni Valley and lower diversity in distant watersheds that harbour Arctic grayling with the same mtDNA lineage.

Contact Zones Among Divergent Lineages

The phylogeographic studies of lake trout and lake whitefish found that contact zones between divergent lineages for both species were located where giant glacial lakes bridged the divide between Arctic and Mississippi watersheds just east of the Rocky Mountains (Bernatchez and Dodson 1991; Rempel et al. 1996; Wilson and Hebert 1998). It is important from an evolutionary perspective to identify these contact zones because secondary contact between divergent lineages often produces sympatric ecotypic polymorphism (e.g. Bernatchez and Dodson 1990; Bernatchez et al. 1996; reviewed in Schluter 1996 and Taylor 1999). Intraspecific divergence can be examined more fully in these areas of contact by observing how the different genotypes partition their environment in sympatry and allopatry. From a conservation perspective, contact zones should be flagged as hotspots with especially high diversity because there may be novel species present that have evolved rapidly (Bernatchez and Dodson 1990; Schluter 1996; Taylor 1999). By examining the phylogeographic structure of Arctic grayling in North America, I will test the hypothesis that contact zones between divergent lineages are concordant among species (Avise 1995) of freshwater fish (e.g. lake whitefish, lake trout and Arctic grayling), giving more credence to certain areas that merit special conservation status or further study.

Arctic grayling in North America survived the Pleistocene glaciation events in Beringia and probably survived in at least one other ice-free region during the Wisconsinan glaciation. What remains unclear is whether there are more than two divergent lineages of Arctic grayling due to survival in up to five isolated ice-free regions, two of which may have been within Beringia itself. The purpose of this chapter is to corroborate the intraspecific phylogeography of Arctic grayling in North America to investigate the evolutionary history behind distinct morphologies (large and small-scale forms) in Alaska, the origin of relict populations in Montana and Michigan (the latter is extinct since 1930's), and the validity of a glacial refuge in the Nahanni Valley. When combined with phylogeography from other species, the information from this study will aid in identifying geographic regions where species and populations are likely to be paleoendemic. Regions with high genetic endemism need to be prioritized for conservation on our rapidly changing planet.

Materials and Methods

Sample Collection

Arctic grayling tissue samples were collected for mitochondrial DNA analysis from 12 regions and 31 populations distributed throughout the species' range in North America (Table 1, Figure 1). For microsatellite analysis, samples came from 26 populations distributed among 10 regions (Table 2, Figure 1). A total of 428 samples (target was 20 fish per population) were analyzed with mtDNA and 629 samples (a target of 30 fish per population) were analyzed with nuclear DNA (microsatellites). Samples consisted of fin tissue that was preserved in 95% ethanol in the field. Some sample sizes were smaller but were still included owing to the importance of the population to the data set (Tables 1 and 2). Sample sizes smaller than 5 and 14 were not used to measure diversity with mtDNA and microsatellites, respectively (Tables 1 and 2). Sample locations and sizes were chosen to determine if Arctic grayling survived glaciation in northern and southern regions of Beringia, upper Missouri River, and the Nahanni Refuge and to find the postglacial dispersal routes used to reach their current distribution. The distribution of the samples was expanded beyond that used by Redenbach and Taylor (1999) to investigate remaining uncertainties from their conclusions. Extensive sampling also took place in the Peace River to investigate the impacts of ecology and geographic barriers on the local population structure of Arctic grayling (addressed more thoroughly in chapters).

DNA Extraction

Total genomic DNA was extracted from the tissue samples using either phenol-chloroform extraction (Taggart et al. 1992) or the PURGENE salt extraction kit. The DNA precipitate was resuspended in TE buffer then stored at -20°C.

Mitochondrial DNA Analysis

The polymerase chain reaction (PCR) was used to amplify 2 adjacent fragments of mtDNA that have been found to be variable in salmonid species (Park and Moran 1994). These fragments were 2100 base pairs in length that included cytochrome b gene and the non-coding D-Loop region, and 2500 base pairs encompassing gene 5 and 6 in the NADH subunit (ND 5/6). Primers for both regions were obtained from published literature: HN20 and Cglu for D-Loop/Cyt-b (Park et al. 1993); C-Leu3 and C-Glu for ND5/6 (Bernatchez and Ozinov 1995). For both fragments the PCR was run in 50µl volumes containing: 800µM dNTP (total), 600nM of each primer, 2 units of *Taq* polymerase along with 1x Gibco BRL reaction buffer and either 3.0 mM $MgCl_2$ (D-loop/cytb) or 2.0 mM MgCl (ND5/6), plus 100 ng of DNA template. PCR parameters were: 5 cycles including 2 minutes at 95°C, 1.5 minutes at 52°C and 1.5 minutes at 72°C, followed by 35 cycles with 1.0 minutes at 94°C, 1.5 minutes at 54°C and 1.5 minutes at 72°C., then finally 5 minutes extention at 72°C. Each PCR product was run on a 1 % agarose gel to confirm the purity and size of the fragment.

Mitochondrial DNA variation was investigated with 15 restriction enzymes (New England Biolabs) after confirming that each enzyme recognized at least one site (recognition sequence) in the Arctic grayling PCR product (Figure 2). Each enzyme was incubated with a mixture of both PCR fragments, then the products were run alongside a one kilobase BRL size standard on 2% agarose gel and stained with ethidium bromide. The two PCR products were digested and run separately for each unique restriction site sequence (fragment pattern) for all enzymes in order to accurately determine the presence or absence of all restriction sites for statistical analysis.

Microsatellite DNA

Polymorphic microsatellite loci in the Arctic grayling genome were located in two ways: by testing published primers that amplify loci found variable in other salmonids and by probing the Arctic grayling genome directly for the presence of tandemly repeating DNA sequences. All primers were first tested with a 'cold' PCR using a gradient of annealing temperatures that ranged 5°C above and below the published or estimated annealing temperature. The PCR products were run on a 2% agarose gel stained with ethidium bromide and viewed under UV light. Successful amplifications consisted of a single bright band that was close to the size of microsatellite locus being tested. Tests for polymorphism and further optimization of PCR conditions were carried out using one ^{32}P end-labeled primer, then running the PCR products on a 6% polyacrilamide gel that was blotted onto filter paper, dried and then exposed to film. These tests for polymorphism were carried out on ten Arctic grayling samples that were selected from a wide geographic range (e.g. Lower Liard River, Yukon River and Upper Peace River), assuming that distinct regions were more likely to show different alleles. Loci that had more than one allele in the test were further optimized and used on the rest of the samples.

A total of sixteen published primers were tested, nine of which did not amplify successfully with PCR: One 2 *(Oncorhynchus nerka,* Scribner et al. 1996), *Ots* 3, *Ots 4 (O. tshawytscha,* Banks et al. 1999), *Ots* 103 (O. *tshawytscha,* Nelson and Beacham 1999), *Ots* 107 (O. *tshawytscha,* Nelson and Beacham 1999), *Sfo* 8, *Sfo* 23 *(Salvelinus fontinalis,* Angers et al. 1995), Sco 1, Sco 2 *(Salvelinus confluentus* , E.B. Taylor unpublished data). Four primers amplified loci but were found monomorphic: Sco 19 *(Salvelinus confluentus,* Taylor et al. 2001), Str 73 *(Salmo trutta,* Estoupe et al. 1993), Ssa 197 *(Salmo salar,* O'Reilly et al. 1996), *Omy 77 (Oncorhynchus mykiss,* Morris et al. 1996). The three primers *One8,* Ots100 and *Bfro04* amplified polymorphic loci in Arctic grayling and were used to analyze samples in this thesis (Table 3).

Novel microsatellite loci were developed for Arctic grayling by first incubating approximately 50 µg of genomic DNA from a single individual with a restriction digest cocktail which contained *Alu* I, *Hae* III, *Mine* II and *Rsa* I. Twenty µg of pUC 19 or Bluescript II SK+ vector was cut with SmaI and dephosphorylated with shrimp alkaline phosphatase (New England Biolabs). The Arctic grayling genomic DNA was run out on a 1.5% low melting point agarose gel in 1X TBE buffer. Restriction fragments between 200 and 800 base pairs were cut out of the gel and purified from agarose using 'beta' Agarase I (New England Biolabs) digestion. Standard cloning procedures of the purified DNA followed Glenn (1995). Positive recombinant clones were picked from the medium with sterile pipette tips, replated onto LB plates and lifted onto nylon hybridization membranes. The recombinant DNA was probed with ^{32}P end-labeled synthetic oligonucleotides $(GC)_{10}$ in Westneat's buffer (7% SDS, 1mM EDTA, 0.263M Na_2PO_4, 1 % BSA) at 60°C. Colony screen positives were picked with sterile pipette tips and stored frozen in sterile distilled water.

The insert DNA was amplified by PCR using M13 primers that had annealing sites located on the plasmid clone flanking the putative Arctic grayling microsatellite DNA. The PCR reactions were carried out in 50µl volumes containing: 5µl of DNA solution from the colony picks, 800 µM dNTP, 1X reaction buffer (Gibco/BRL), 3.0mM $MgCl_2$ and 5pM each of the M13 primers (forward; 5'-TGTAAAACGACGGCCAGT-3'; reverse, 5'-CAGGAAACAGCTATGACC-3'). The PCR reactions entailed 25 cycles of 94°C denaturing for 1 minute, 50°C annealing for 1 minute and 72°C extension for 1.5 minutes. PCR products were purified for sequencing using 'Qiaquick' PCR purification columns(QIAGEN), eluted into 50µl of distilled water and delivered to the sequencing lab for processing. If tandemly repeating DNA was present in the sequence, primers were designed to flank the microsatellite region. The program 'Primer 3' was used to find the best primer sequences that recognized unambiguous sites and had similar annealing and melting temperatures. A total of twenty-seven screen positives were sequenced and examined for presence of tandemly repeating DNA. Of these ten had tandem repeats for which flanking primers could be made and only two microsatellite loci were found to be polymorphic in the Arctic grayling (Table 3).

PCR reactions for each optimized microsatellite locus (Table 3) were carried out in 10µl volumes containing: 100ng DNA template, 1X reaction buffer (Gibco/BRL), 400 µM dNTP, 1.5 mM $MgCl_2$, 600nM unlabelled primer, 300nM ^{32}P end-labeled primer which consisted of 50nM from endlabelling reaction plus 250nM added directly to the PCR. Standard PCR conditions used were: 1 cycle at 95°C denature for 3 minutes, 5 cycles at 94°C denature for 1 minute, hot annealing temperature (see Table 3) for 1 minute, 72°C extension for 1 minute; 30 cycles at 92°C denature for 45 seconds, optimum annealing temperature (see Table 3) for 1 minute, 72°C extension for 1 minute, 1 cycle at 72°C final extension for 5 minutes. The PCR products were run on a 6% polyacrylamide gel along with a ddNTP ladder, and the loci were scored by eye from exposed film.

Data Analysis

The fragment patterns generated by the restriction enzyme digests were used to infer presence or absence of a restriction site for each sample. The restriction site data was converted to binary (1, 0) character matrix format that was imported into the Restriction Enzyme Analysis Package (REAP, McElroy et al. 1992). This was used to calculate the number of nucleotide substitutions per site, *d* (Nei and Tajima 1981; Nei and Miller 1990), weighted by the class of restriction enzymes used (Nei and Tajima 1983). Haplotype diversity (Nei 1987) and nucleotide diversity (Nei and Tajima 1981) within populations and nucleotide divergence between all population pairs (Nei and Tajima 1981) were also determined using DA program from REAP. A parsimonious mutational network was generated by eye from the restriction enzyme site profiles for each haplotype.

Cluster analysis among haplotypes and populations was carried out using PHYLIP version 3.57c (Felsenstein 1995). The restriction site matrix was imported into PHYLIP and replicated 1000 times using SEQBOOT, then a most parsimonious tree was built from these replicate data sets using Wagner MIX parsimony and the CONSENSE programs in PHYLIP. Nucleotide divergence estimates among haplotypes (output file from the *d*, Nei and Miller 1990) and nucleotide divergence estimates from all population pairs (output file from DA, Nei and Tajima 1981) were importing into PHYLIP from REAP to generate cladograms. The unweighted pair group method with arithmetic means cluster analysis (UPGMA; Sneath and Sokal 1973) and the Neighbor-Joining Method (Saitou and Nei 1987) were both used to build trees due to the different assumptions of evolutionary rates among populations. The UPGMA cluster analysis uses a phenetic approach, which assumes equal evolutionary rate among all operational taxonomic units (OTU's). Nevertheless, tree topologies have been recovered well in empirical tests, due to large stochastic error in genetic distance estimates (Avise 1994). The Neighbor-Joining Method assumes variable evolutionary rates among OTU's and could reveal a true topology and realistic branch lengths if the genetic distance estimates are a good representation of empirical evolution.

Descriptive statistics of microsatellite loci included expected and observed heterozygosity, average number of alleles per locus, Hardy Weinberg Equilibrium exact tests, all calculated using the GENEPOP program version 3.1 (Raymond and Rousset 1995). Genetic distance estimates were calculated by creating a microsatellite allele frequency matrix and replicated 1000 times with SEQBOOT from PHYLIP. Genetic distance estimates were calculated for each replicate data set using GENDIST program and consensus trees were built all with the PHYLIP analysis program. Both Cavalli-sforza chord distance (Cavalli-sforza and Edwards 1967) and Nei's original distance (Nei 1972) were estimated from the data.

Cavalli-Sforza's chord distance (C-S chord distance; Cavalli-sforza and Edwards 1967) assumes no mutation and Nei's original distance (Nei 1972) assumes the infinite alleles model of mutation. Both distance estimates assume population differentiation results from genetic drift although Nei's distance assumes constant and equal population size. C-S chord distance is not constrained by this assumption, thus allowing for different rates of population divergence over time due to drift. A third measure of distance, Goldstein's $\delta\mu^2$ distance (Goldstein et al. 1995) assumes stepwise mutation model, which has been suggested for microsatellites (Jarne and Lagoda 1996). Beebee and Rowe (1999) compared these three genetic distance measures to describe the phylogeographic structure of the natterjack toad *(Bufo calamita)* in Europe and found C-S Chord distance gave the best description of differentiation among populations that were founded from a single eastern European glacial refuge. This was due to an overwhelming influence from genetic drift over mutation, in populations that were isolated since glaciation ended, and variable divergence rates among populations that resulted from naturally variable population sizes.

Demographic processes probably overwhelm mutation based differentiation among populations that were founded postglacially (Paetkau 1997; Beebee and Rowe 1999; Taylor and McPhail *in press)*, given the time since colonization (~12,000 years) and fluctuating population sizes. Nevertheless, mutations might be an increasingly important consideration for measuring genetic divergence among populations from distinct glacial refugia. Considering that unique alleles increase Nei's genetic distance estimates, this measure might be better than C-S chord distance for clustering populations originating from different glacial refugia. A number of empirical studies have found that the infinite alleles model (IAM) of mutation describes population structure better than the stepwise mutation model (SMM) at various time scales (Angers and Bernatchez 1997; Garcia de Leon et al. 1997; Orti et al. 1997; Paetkau et al. 1997; Estoup et al. 1998; Mutter et al. 1998). This is likely because new alleles are generated at microsatelite loci through a process that is more complex than a simple stepwise mutation model (Ellegren 2000). Consequently, I employed genetic distance estimates from the microsatellite allele frequencies that assume the IAM of mutation (e.g. Nei 1972). Both UPGMA and N-J method were used for cluster analysis of both C-S distance and Nei's original distance (see above).

Partitioning of the genetic variance of both mtDNA and microsatellite loci among populations and among distinct geographic regions was analyzed with analysis of molecular variance (AMOVA; Excoffier et al. 1992). MtDNA RFLP site matrix and haplotype distribution, and allele frequency for microsatellite loci were imported into the ARLEQUIN program (Schneider et al. 1997) for these analyses.

Results

Mitochondrial DNA Variation Among Lineages

Mitochondrial DNA diversity and phylogenetic relationships among haplotypes

Restriction enzyme analysis of Arctic grayling mitochondrial DNA surveyed 103 restriction sites and 446 base pairs, about 9.6% of the ND 5/6, cytochrome b/D-loop fragment. Average pairwise nucleotide divergence among haplotypes was 0.92% and ranged from 0.1% to 2.3%.

Cluster analysis of sequence divergence estimates among the haplotypes distinguished three groups from the data (Groups A, B and C, Figures 3 through 5). Generally all three methods clustered haplotypes 7 through 12 with strong bootstrap values (100, 97 and and 83 for Wagner parsimony, Neighbor-Joining and UPGMA, respectively). Haplotypes 11 and 12 consistently constituted group C with high bootstrap values while only UPGMA clustered haplotypes 7 through 10 into group B with strong bootstrap support. The remaining haplotypes (1 through 6) made up group A although there was low bootstrap support for these haplotypes as a clade. Nevertheless, haplotypes 3 through 6 tended to cluster together consistently in each of the trees although bootstrap values were always low (Figures 3 through 5). Generally within group A, low bootstrap values among the haplotypes suggest that they arose independently from an ancestal haplotype that was not included in the data set. The clustering of haplotypes 3, 4, 5 and 6 was due to common sites recognized by *Hinf* I (Tables 4 and 5, Figure 2) suggesting that these haplotypes arose in Arctic grayling populations that were isolated from the rest of group A.

Average nucleotide divergence estimates among the haplotype groups was greatest between groups C and A and was lower but still relatively high between groups C and B (Table 6). This is a reflection of 21 restriction site changes that occurred between groups C and A and 14 changes between groups C and B (Figure 2). The parsimony mutation network analysis suggests that group B (haplotypes 7 through 10) is ancestral to both other groups (Figure 2), especially with reference to Redenbach and Taylor (1999) where haplotypes 7 and 8 (their haplotype G) were ancestral when a *T. thymallus* haplotype was used to root their tree. The likelihood of haplotypes 7 and 9 being ancestral to group C was equal but haplotype 7 was chosen due to its geographic distribution relative to group C (see below). Both nucleotide divergence estimates and the parsimony network suggest that group C is distinct from groups A and B (Table 6, Figure 2) but cluster analysis distinguished groups B and C separately from group A (Figures 3 to 5). Examination of the site matrix, however, reveals that groups B and C have similar recognition sites with *Alu I*, *Rsa* I, *BstU* I, *Dde* I and *Hha* I suggesting they were derived from the same common ancestor (Table 4 and 5). Groups A and C show site changes with the same restriction enzymes *(Dpn* II, *Hae* III and *Hinf* I) but the site matrix shows they involve different site changes within the recognition sequence, suggesting that these groups diverged independently (Table 4).

Cluster analysis among Arctic grayling haplotypes including North American, Siberian and the European grayling *(T. thymallus)* suggests that group C haplotypes are ancestral to groups B and C (Figure 6). Only eight restriction enzymes (the first eight listed in Table 4) were used to generate this tree so haplotypes 2 (group A) and 7 and 8 (group B) were not distinguished and haplotype groups A and B were no longer distinguished with high bootstrap support (Figure 6). Nevertheless, there was statistical support (bootstrap of 76%) supporting divergence of group C haplotypes from a common ancestor with all Beringian and Siberian haplotypes examined.

Mitochondrial DNA haplotype distribution and population differentiation within and among assemblages.

Average haplotype diversity and average nucleotide diversity within populations were 0.1962+/-0.00211 and 0.00217+/-0.0000009, respectively. Average nucleotide divergence estimates and diversity estimates among populations in the data set were 0.005844+/-0.0000001 and 0.008015+/-0.0000001, respectively. Both haplotype diversity and nucleotide diversity were greatest in populations from more northern latitudes (Table 1, Figure 7).

Haplotypes in group A (1 through 6) were distributed among populations from Northern Alaska, along the north Pacific Coast to the Peace River in British Columbia (Table 5, Figure 7). Two Pacific coast drainages, Copper River (N=18) and Becharof Lake (N=10) were investigated with *Alu* I and *Bst*U I that were diagnostic for sites within the ND 5/6 fragment and established their designation into group A. This diagnostic analysis also found that 10 individuals in the Niukluk River in the Seward Penninsula belonged to group A. Haplotype 1 was by far the most common (183 individuals, 49% of the whole data set) and was widely distributed from the lower Yukon River in Alaska to the lower Peace River and lower Liard River in British Columbia (Table 5, Figure 7). The remaining haplotypes in group A were generally more locally distributed in interior Alaska and northern British Columbia (Table 5, Figure 7). Given the distribution of these haplotypes in the southern and central areas of Beringia, I have desigated group A haplotypes as "South Beringia" (Figure 7).

Group B (haplotypes 7 through 10) ranged most widely, from northern Alaska into the lower Mackenzie River, south to the upper Missouri River, and as far east as Wollaston Lake, Saskatchewan (Table 5, Figure 7). Haplotypes 8, 9 and 10 were predominant in the north and haplotype 7 was the only one found in the south (Table 5, Figure 7). Diagnostic analysis of the Niukluk River (mentioned above) designated 11 group B individuals in the Seward Peninsula (Table 5). Interestingly, there were no group B haplotypes found in other populations located between the northern and southeastern extremes of the geographic range of this group (eg. Great Slave Lake or lower Liard River, Table 5, Figure 7). Although these haplotypes are widely distributed, the greatest haplotype diversity within this group was found on the north slope of Beringia (Beaufort Sea, Table 1, Table 5, Figure 7). I have therefore designated group B haplotypes as "North Beringia" (Figure 7).

Group C consisted of only two haplotypes (11 and 12) and their geographic range did not extend beyond the lower Mackenzie drainage (Table 5, Figure 7). Given the close association with the Nahanni River (a tributary of the lower Liard), I have designated group C haplotypes as "Nahanni lineage".

In summary, the three mitochondrial DNA lineages were distributed among four distinct regions within the species' range (Figure 7). The South Beringian lineage (group A haplotypes) were found in the northwest among the Seward Peninsula, Yukon River, Pacific Coast drainages, upper Liard River and Peace River. The Nahanni lineage (group C haplotypes) was found in the Mackenzie River as far west as the lower Liard River and as far east (upstream) as Great Slave Lake. The North Beringian lineage (group B haplotypes) were split among two regions, one in Arctic Alaska (the Beaufort Sea coast and Seward Penninsula) and one in the southeast interior in Saskatchewan (Wollaston Lake and Black Lake) and Montana (upper Missouri River). Nahanni Arctic grayling appear to have bisected the distribution of North Beringian Arctic grayling at the middle section of the Mackenzie River.

Populations in the lower Liard had both group A and group C haplotypes and populations in the Seward Peninsula, lower Yukon River and lower Peace River had both group A and group B haplotypes (Table 5, Figure 7). The greatest level of mitochondrial DNA lineage mixing occured in the lower Liard River (LaBiche, Beaver and Minnaker rivers) and Seward Peninsula (Niukluk River) where group A haplotypes were found in the same populations with group C and group B haplotypes, respectively. Although they were rare, group B haplotypes were also found in the lower Yukon River (Chena River) and the lower Peace River (Burnt River) where group A haplotypes were predominant. There were no examples in the data set where both group B and group C haplotypes occurred together in one population.

Microsatellite Variation Among Geographic Areas.

Microsatellite allele frequency data were transformed to correct for significant heterozygote deficiencies that resulted from differential primer specificity among alleles at three loci. Null alleles were observed for *Tar* 1 and *Ots* 100 loci and there were significant heterozygote deficiencies in the populations where these null homozygotes were observed (Fisher's exact test, $p<0.05$). Similarly, the most common allele (196 bp) at *Tar 8* locus was only observed as a homozygote in populations where other alleles were present. Null heterozygotes were estimated using maximum likelihood for all populations containing null homozygotes at *Tar* 1 and *Ots* 100 loci and for all populations that had more than one allele at *Tar 8* locus (Dempster *et al.* 1977). This transformation corrected for heterozygote deficiencies in most populations although a few remained for *Tar* 1 and *Tar 8* as well as for *BFRO 4* (Table 2). These departures from Hardy Weinberg expectations (HWE) did not occur across more than one locus within any population, nor were they frequent across populations within one locus

(Table 7) so these data were used for further analysis of diversity and divergence among populations.

Genetic diversity among populations

There were noticeable differences in the number of alleles within populations (Table 2). Genetic diversity (mean number of alleles and expected heterozygosity) declined remarkably with geographic distance from the middle Yukon River in Alaska (Figures 8 and 9). The middle Yukon River is located in central Beringia, which remained ice free throughout the Pleistocene Epoch. Arctic grayling most likely survived glaciation then expanded their range postglacially from this region (see introduction). Among all populations there was a significant negative correlation between geographic distance and the mean number of alleles per locus ($r = -0.424$, $0.025<p<0.01$). The correlation coefficient was greater ($r = -0.772$, $p<0.0005$), however, among "South Beringian" populations (containing mtDNA lineage A) located in southern interior Alaska, the Yukon and British Columbia (see Figures 8 and 9). This was due to removing genetically diverse populations that were located near the "Nahanni" and "North Beringian" glacial refugia and that contained mtDNA lineages C and A respectively and could obscure the signature of dispersal from the Yukon River valley (Figure 8). Although expected heterozygosity also declined with geographic distance from Beringia, the correlation coefficient was not significant among all sampled populations ($r = -0.123$, $p>0.125$) but it was larger and significant among "South Beringian" populations ($r = -0.588$, $0.0025<p<0.005$). A weaker correlation coefficient with this measure of diversity was likely due to differences in allele frequencies among the populations.

Genetic divergence among populations

Compared to the Neighbor-Joining method, UPGMA generated trees that corresponded more closely to that expected from mtDNA results and the hypothesized zoogeography (Figure 10: A & C versus B & D). The N-J method (Figure 10 B and D) did not reveal any striking population clusters although there were higher bootstrap values with Nei's genetic distance. The UPGMA of both Cavalli-Sforza chord distance and Nei's original distance gave bootstrap support for distinguishing three Nahanni origin populations (containing group C mtDNA haplotypes) as a group (Figure 10 A and C). Only the UPGMA of C-S chord distance however, clustered the Great Slave Lake population with the other Nahanni populations (Figure 10 A). There was also bootstrap support deep within this tree (61%) that distinguished the Nahanni and most Beringian origin populations separately from two populations located in Saskatchewan (Wollaston Lake) and the lower Peace River (Beatton River) in British Columbia (Figure 10 A).

Genetic divergence among populations measured with microsatellite loci only partially corresponded with the evolutionary history inferred from the geographic locations of monophyletic groups of mtDNA (Figure 10 A). Both genetic distance estimates (Nei's original distance and C-S Chord distance) found low divergence among populations located in Alaska, the Yukon and British Columbia, even though there were two monophyletic groups of mtDNA (groups A and B) found in the region (Figure 10 A). Furthermore the most divergent populations (measured with microsatellites) contained mtDNA group B, but were geographically isolated in Saskatchewan (Wollaston Lake). Populations located in the lower Liard and Great Slave Lake clustered together at an intermediate genetic distance from the other two groups although they were all predominantly the most divergent mtDNA group C (Figure 10 A).

There were no fixed allele differences that were associated with glacial refugial groups of Arctic grayling in North America although several unique alleles were found (Figure 11 A through E). Alleles unique to the south Beringian lineage were found in Alaska (Becharof Lake: Tar 1, allele 105, Koyukuk River: One 8 alleles 167, 171, 173) and British Columbia (Atlin Lake: One 8 allele 167)(Figure 11 A and D). Unique alleles for the Nahanni lineage were found in Great Slave Lake *(Tar* 1 allele 87, One 8 allele 179) and in the LaBiche and Beaver Rivers (One 8 allele 179)(Figure 11 A and D). The populations within the north Beringian lineage were divided according to their geographic location to the north and south of the Nahanni populations (see Figure 8), and unique alleles were found associated with both regions. In the Alaskan populations most of the unique alleles were found in the Seward Peninsula (Niukluk River, *Tar 8* allele 186, *Ots* 100 the null allele, *BFRO* 4 allele 172), but one was also found on the Beaufort Sea coast (Sagavonirktok River, *Tar 8* allele 190)(Figure 11 B, C and E). All of the unique alleles in the southern part of the lineage's range were found in Black Lake, Saskatchewan (One 8 alleles 183 and 187)(Figure 11 D).

There were also allele frequency differences found among the glacial refugial groups (Figure 11 A through E). The south Beringian and the Alaskan north Beringian populations had the same most frequent alleles at all loci but the 'vicariant' north Beringian group (Saskatchewan populations) had different alleles that were most frequent at *Tar* 1, *Ots* 100, *One 8* and *BFRO* 4 (Figure 11 A, C, D and E). The most frequent alleles in the Nahanni populations were different from all other refugial groups at Tar 1, *Tar 8,* and BFRO 4 (Figure 11 A, B, and E). There were similarities however, between Nahanni and the 'vicariant' north Beringian group at *Ots* 100 (Figure 11 C), and between Nahanni and the South Beringian, and North Beringian groups at One 8 (Figure 11 D). Other alleles were common throughout Beringian and Saskatchewan populations (eg. *Tar* 1 the null and 79 alleles), but were only found in one Nahanni population (Great Slave Lake) suggesting limited gene flow with Nahanni. Only one allele was common in north Beringia and Saskatchewan, but rare in south Beringia and not present in Nahanni populations *(BFRO 4* allele 68, Figure 11 E).

Geographic pattern of within population diversity

Within the whole data set, 85% of the mtDNA variance occurred among populations and 15% was accounted for within populations (F_{st} = 0.85, AMOVA). UPGMA cluster analysis of nucleotide divergence estimates among populations (DA from REAP) revealed three groups that corresponded to the most frequent mtDNA lineage present within the population groups (Figure 12). Relative to other hierarchies tested, partitioning of genetic variance among these putative refugial groups accounted for the greatest proportion of the total variation found among regions and the least amount of variation within populations (Table 7). Differentiation among populations within regions was also greater than when other hierarchies were tested (F_{st}= 0.91, Table 7).

Analysis of genetic variance carried out for microsatellite allele frequency data found results similar to mtDNA, although differentiation between 'north Beringian' populations located in northern Alaska and Saskatchewan was apparent with microsatellite data (Table 8). In contrast to the mtDNA results, genetic variation among groups was greatest when the Saskatchewan populations were treated as a separate group, even though they contained the same mtDNA lineage (mtDNA B) to other north Beringian populations located in Alaska (Table 8, Figure 7). Among all of the samples, 41% of the genetic variance was accounted for among populations and 59% occurred within populations (F_{st}= 0.41, AMOVA).

Measures of genetic diversity from nuclear DNA and mtDNA were positively and significantly correlated with each other (Figure 13). The correlation coefficient was highest between measures derived from the mean number of alleles per locus (microsatellite DNA) and haplotype diversity (mtNDA) ($r = 0.584$, $0.002<p<0.005$). Although they were also positive, the correlation coefficients between expected heterozygosity and mtDNA diversity were lower (nucleotide diversity: $r = 0.294$, $0.10<p<0.20$, haplotype diversity: $r = 0.542$, $0.005<p<0.01$; not shown in Figure 13). The correlation coefficient between mean number of alleles and nucleotide diversity was: $r = 0.316$, $0.10<p<0.20$. These results suggest that the mean number of alleles per locus and haplotype diversity have been affected by historical events in more similar ways than either expected heterozygosity or nucleotide diversity.

Discussion

The distribution of genetic diversity among North American Arctic grayling populations showed a remarkable resemblance to expectations from the models proposed by Hewitt (1996) and Ibrahim (et al. 1996). My data found three mtDNA lineages and each was distributed geographically such that the greatest genetic diversity was located in regions that remained ice-free during the Wisconsinan glaciation (Figure 7). MtDNA haplotype groups A and B were distributed in Alaska remarkably similar to the large and small scale sized morphological phenotypes that were discovered by McCart and Pepper (1971). Together these data support the hypothesis that Arctic grayling survived glaciation in two isolated regions in Beringia. The small-scale form and haplotype group A were located in the south, which suggests they survived glaciation in the ice-free portion of the Yukon River. The large-scale phenotype and haplotype group B were both located in the north, which suggests they survived glaciation in Arctic Beringia, north of the Brooks Range. The local distribution of haplotype group C around the Nahanni Valley supports the hypothesis that a third group of Arctic grayling survived the Wisconsinan glaciation in the Nahanni Refuge. Postglacial range expansion for the Nahanni grayling, however, was limited to watersheds within the Mackenzie drainage. Such evidence for limited dispersal from the Nahanni Valley does not support the hypothesis that the location of the Nahanni refuge offered easy access to giant glacial lakes (e.g. Glacial Lake Peace, Glacial Lake Agassiz), which facilitated range expansion for freshwater fish in North America (Lindsey and McPhail 1986). Furthermore, my data found no evidence that Arctic grayling survived the Wisconsinan glaciation in the Mississippi Refuge: the hypothesized origin for relict populations that once inhabited watersheds in Michigan (McPhail and Lindsey 1970; Lindsey and McPhail 1986).

A disjunct distribution of the group B mtDNA lineage in North America, with high genetic diversity along the Arctic coast and low genetic diversity in Montana and Saskatchewan (Figure 7, 8), supports the hypothesis that early Wisconsinan dispersal was possible for freshwater fish between the Cordilleran and Laurentide ice sheets. Arctic grayling expanded their range southward from the Beringian Arctic coast then became isolated by advancing glaciers and survived the late Wisconsinan near the upper Missouri River (which explains the fossil in southern Alberta; Burns 1991). Subsequently, these Missouri refuge grayling founded (or remain as) relict populations in the upper Missouri River (Montana), but they also expanded their range into the northeast at least as far as Saskatchewan. The distribution and genetic diversity within mtDNA group A (Figure 7, 9) supports the hypothesis that postglacial range expansion from the Yukon River (south Beringia) was limited by the persistent Cordilleran ice sheets.

Regional partitioning of molecular variance of two independent genetic markers, mtDNA and nuclear DNA gave further support for the hypothesis that Arctic grayling survived in three glacial refugia during the Wisconsinan glaciation: north Beringia (Brooks Range refuge), south Beringia (Yukon River refuge) and the Nahanni Refuge (Table 7, 8). The AMOVA from microsatellite loci (Table 8), however, supports the upper Missouri River refuge hypothesis because greater genetic variation at microsatellite loci gave greater precision for distinguishing among population groups derived from a more recent common ancestor. This concurring distinction among groups of Arctic grayling populations using two independent DNA markers gives rigorous support to the hypothesis that distinct mtDNA lineages (groups A, B and C) represent refugial groups of Arctic grayling. Such evidence refutes the hypothesis that stochastic lineage sorting resulted in the current distribution of mtDNA lineages and that Arctic grayling reached their current geographic range through range expansion from a single glacial refuge (e.g. Beringia).

Further corroborating evidence (geological and genealogical) that give further support for these hypotheses are discussed below.

Divergence among Arctic grayling mtDNA lineages

Average nucleotide divergence among Arctic grayling haplotypes (0.92%) was similar to the average intraspecific divergence found for other freshwater fish species from glaciated regions (0.9%; Billington and Hebert 1991 cited by Bernatchez and Wilson 1998). Maximum divergence, however, was considerably higher than the average maximum intraspecific divergence for 25 north temperate freshwater fish species (2.3% compared to 1.2% respectively; Bernatchez and Wilson 1998). Levels of divergence between Arctic grayling haplotype groups A and B are within the range of intraspecific divergence, but the divergence of group C haplotypes from the other two groups (Table 6) is comparable to levels of divergence between sister-species of freshwater fish (ranges from 1.2% to 6.2%; Bernatchez and Wilson 1998). In fact, compared to recognized sister species within the genus *Coregonus* (ranged from 1.2% to 1.8%; Bernatchez and Wilson 1998), the Nahanni grayling are highly divergent from other North American Arctic grayling (Table 6).

Levels of divergence among the three Arctic grayling haplotype groups (Table 6) were substantially lower than divergences found among three European grayling *(T. thymallus)* haplotype groups, which ranged between 3.11% to 4.55% for the same mtDNA region (D-loop/Cyt b, ND 5/6; Koskinen et al. 2000). There were also substantially more haplotypes found in the European *T. thymallus* populations (27 haplotypes from 540 individuals, among 27 populations; Koskinen 2000) relative to North American *T. arcticus* populations in my study (12 haplotypes from 320 individuals, among 31 populations). These differences in depth of divergence and haplotype diversity between the species suggest North American Arctic grayling were more severely impacted by Pleistocene glaciation events than European grayling. Although the phylogeographic structure of *T. arcticus* in North America and *T. thymallus* in Europe both found three refugial groups, greater diversity and divergence among the groups implies *T. thymallus* suffered fewer extinctions during the Pleistocene and persisted at larger effective population sizes for longer periods in ice free regions of Europe.

Lake whitefish *(Coregonus clupeaformis* and *C. lavaretus)* were similarly impacted less severely by Pleistocene glaciations in Europe relative to North America (Bernatchez et al. 1989; Bernatchez and Dodson 1994; reviewed in Bernatchez 1995 and Bernatchez et al. 1999). Although there were similar numbers of haplotypes found on both continents, haplotype diversity was distributed more evenly among populations throughout northwestern Europe. In North America, however, diversity was concentrated in historically ice-free regions in the southeast (Mississippi and Atlantic refugia) and northwest (Beringia) (Bernatchez et al. 1989; Bernatchez and Dodson 1991, 1994; Bernatchez et al. 1999). Higher levels of diversity in European populations were attributed to a smaller extent and lesser impact of Eurasian ice sheets relative to

North America (Bernatchez and Dodson 1994).

The lake whitefish species complex, however, had shallow divergence among haplotype clades (ranged from 0.4% to 1.2%; Bernatchez et al. 1999) relative to clades within *T. arcticus* (ranged from 0.75% to 2.1%; Table 6) and within *T. thymallus* (3.1% to 4.6%; Koskinen et al. 2000). Furthermore, the lake whitefish mtDNA clades were distributed from northwestern Europe to northeastern North America (Bernatchez and Dodson 1994) while all five grayling species are located in distinct regions in North America and Siberia *(T. arcticus* Pallus, 1776), Mongolia *(T. nigrescens* Dorogostajskij, 1923; *T. grubei,* Dybowski 1869; *T. brevirosths,* Kessler 1879) and Europe *(T. thymallus* Linneus 1758) (Schoffmann 2000). Locally distributed clades and greater divergence within and among *T. thymallus* and *T. Arctic's* suggests that Pleistocene range expansion for *Thymallus* species was more limited relative to *Coregonus* species.

Clustering among Arctic grayling haplotypes determined with eight restriction enzymes suggests that the Nahanni haplotypes (group C) are ancestral to Beringian and Siberian (Kamchatka) haplotypes (Figure 6). Also, cluster analysis of divergence estimates (Figure 3, 4, 5) and the parsimony network (Figure 2) among the twelve haplotypes found with 15 restriction enzymes indicated that the group B (north Beringian) haplotypes were more closely related to group C (Nahanni). Together, these data suggest that group B haplotypes are ancestral to group A (south Beringia).

Founding and postglacial dispersal of the Nahanni lineage in North America

My data indicate that the most divergent lineage of North American Arctic grayling survived glaciation in the interior of North America (Nahanni Refuge). Arctic grayling probably became established in the Nahanni region prior to the onset of the Wisconsinan glaciation, but the question remains: when did they arrive in North America? Arctic grayling probably first crossed the Bering land bridge from Siberia as early as the mid Pliocene or the beginning of the Pleistocene (≥2 million years ago; Makoedov 1987), although there were multiple opportunities for fauna exchange throughout the Pleistocene (Lindsey and McPhail 1986). Of the three lineages of North American Arctic grayling identified in this study, the Nahanni group probably descended from the earliest colonizers of North America. This is largely based on the amount of genetic divergence from the other North American lineages (about 2% sequence divergence from group A and about 1.6% from group B) relative to the divergence of the Kamchatkan Arctic grayling from North American groups A and B (about 1 % sequence divergence, Figure 6). Also the local distribution of mtDNA group C in the Mackenzie drainage and its absence from Beringia suggests distant North American ancestry. These data suggest that the mtDNA lineage C has been isolated from the other North American mtDNA lineages for most of the Pleistocene (1 to 2 million years, Brown et al. 1979; Bernatchez and Dodson 1991; Smith 1992).

If this is an accurate interpretation then it appears that the Nahanni lineage is slowly being replaced by the two lineages that survived the last glaciation (Wisconsinan) in Beringia, and currently enjoy a wider distribution (Figure 7). A feasible alternative, however, is the Nahanni lineage is a vicariant group with a close relative located in Siberia, where grayling are abundant. Freshwater dispersal routes between northeast Asia and northern Alaska were available (see below) so a genetically divergent group could have colonized the Mackenzie River during the Pleistocene. Although Nahanni Arctic grayling are most likely endemic to North America, this needs to be confirmed with mtDNA analysis of northeast Siberian Arctic grayling.

Geological (Ford 1976, Bodaly and Lindsey 1976) and genealogical evidence (Lindsey and McPhail 1984, Foote et al. 1992, Wilson and Hebert 1998) hints at two distinct regions located between the Cordilleran and Laurentian ice sheets (just west of the Mackenzie drainage) where glacial lakes persisted during Wisconsinan glaciation. The southern most refuge included a large northward draining glacial lake that filled the lowlands of the South Nahanni River and North Nahanni River (Ford 1976) and has been called the Nahanni Refuge. These streams resumed their current flow into the Liard River and Mackenzie River soon after the glaciers retreated. The northern refuge included glacial lakes that flooded portions of the Peel and Porcupine drainages that drained west into the Yukon watershed (Bodaly and Lindsey 1977, Lindsey and McPhail 1986) but my data support the existence of the Nahanni refuge. For instance, all of the Arctic grayling populations that contain mtDNA lineage C are locally distributed around the putative Nahanni refuge and no dramatic decline in genetic diversity among the populations suggests few founder events from postglacial dispersal. A single sample located further downstream in the Mackenzie, closer to the Peel River were all lineage B (Keel River, Table 5) suggesting a north Beringian origin. Furthermore, Arctic grayling with the large scale phenotype that was associated with mtDNA group B were also associated with Peel River freshwater fish fauna (Bodaly and Lindsey 1976, McCart and Pepper 1970). Dispersal downstream through the Mackenzie River from the Nahanni may have been limited because Arctic grayling from north Beringia had already filled the habitat in the lower reaches of the drainage.

Both lake whitefish *(Coregonus clupeaformis,* Foote et al. 1992) and lake trout *(Salvelinus namaycush,* Wilson and Hebert 1998) have mtDNA lineages that are associated with the Nahanni Refuge. Both lineages, however, have a wider geographic range than Arctic grayling in the Nahanni area and in North America in general. Survival of lake whitefish and lake trout in eastern refugia (Bernatchez and Dodson 1991; Wilson and Hebert 1998) probably facilitated postglacial dispersal by these species in giant glacial lakes (McPhail and Lindsey 1970; Lindsey and McPhail 1986; Crossman and McAllister 1986; Dyke and Prest 1987). Arctic grayling, by contrast, are most often found in streams, especially in the northwest and near the limits to their geographic range (Armstrong 1980; Northcote 1995). This dependence on fluvial habitat may have limited their postglacial dispersal through glacial lakes.

Founding of Arctic grayling in Beringia

The distribution of mtDNA lineage B in northern Alaska and the Mackenzie River (Figure 7) is remarkably similar to the distribution of the large lateral line scale phenotype of Arctic grayling (McCart and Pepper 1971), suggesting they both evolved in isolation in northern Beringia. Further, similar biological characteristics (Chereshnev 1990; Skopets 1991) and colour and pattern of the dorsal fin (Makoedov 1987) suggest that Arctic grayling on the Chukotka Peninsula and Arctic Alaska originated from the same Beringian drainage that was likely located in the north slope lowlands of Beringia (Chuckchi Sea River). Phylogeographic evidence from lake whitefish (Bernatchez and Dodson 1991, 1994) and Arctic char *(Salvelinus alpinus,* Wilson et al. 1996) and geological evidence (Hopkins 1972 cited by Lindsey and McPhail 1986) suggest that dispersal routes between North America and Siberia were available for freshwater fish during Wisconsinan and Illinoian glaciations. Drainage channels are still present on the bottom of the Chuckchi Sea that are a testament to an extensive drainage of the Beringian north slope lowlands linking the Chukotka Peninsula in Siberia with the Seward and St. Lawrence peninsulas in Alaska during Pleistocene glaciations (Lindsey and McPhail 1986; Skopets 1991). The Eurasian lake whitefish clade most likely used this drainage connection to disperse between northwestern North America and northeastern Siberia. Likewise, Arctic grayling bearing mtDNA lineage B likely expanded their range through the north slope of Beringia, although further mitochondrial DNA analysis from Chukotka Peninsula is needed to confirm that Arctic grayling (and Arctic char, Wilson et al. 1996) from northern Alaska and Chukotka Peninsula have common ancestry (mtDNA group B).

Mitochondrial DNA groups A and B probably evolved in isolated regions in the south and north slopes of Beringia, respectively. The amount of genetic divergence between mtDNA groups A and B (0.7% sequence divergence; Table 4) suggests they diverged up to 700,000 years ago, before the beginning of the Illinoian glaciation (Lindsey and McPhail 1986). There is geological evidence that north and south slope freshwater drainages in Beringia were isolated from each other for at least 130,000 years, until the end of the Sangamon interglacial. Isolation between these regions of Beringia was more complete during Illinoian glaciations relative to the Wisconsinan due to greater glacier formation and longer glaciation (~50,000 years between 170,000 to 120,000 years ago, Hopkins 1973 cited by Lindsey and McPhail 1986). The Wisconsinan glaciation was more erratic with various intervening warm interstadial periods that allowed freshwater fish to move among Beringian drainages between about 50,000 and 20,000 years ago (Lindsey and McPhail 1986). These interstadials may also have opened dispersal corridors for freshwater fishes between the Cordilleran and Laurentide ice sheets just east of the continental divide. A viable hypothesis for the divergence between mtDNA groups A and B is that Arctic grayling were founded in the south slope of Beringia before the Illinoian glaciation (between 700,000 and 350,000 years ago) and remained isolated throughout the Illinoian glaciation and possibly until the end of the Sangamon glaciation. Alternatively, both haplotype groups A and B arrived in Beringia at the same time within the same population, and their current distribution in Alaska resulted from stochastic lineage sorting as Arctic grayling expanded their range postglacially in North America.

My data, however, support the former hypothesis. If the assortment of mtDNA groups A and B reflect recent (postglacial) random events, then there would not be corroborating evidence from the microsatellite DNA, given the relatively large effective population size and random assortment of nuclear loci across generations. In fact, the least amount of variance at microsatellite loci was accounted for among regions (18%, Table 8) when all populations west of the continental divide (all bearing haplotype groups A and B) were treated as a single group. The greatest proportion of genetic variance at both nuclear and mtDNA loci were accounted for among regions when the populations were divided according to their putative refugial groups (Table 7, 8).

Considerable genetic exchange among Beringian origin populations of Arctic grayling was evident from mtDNA haplotype distribution (Figure 7) and microsatellite DNA estimates of population divergence (Figure 10). Opening and closing of the early Wisconsinan land bridge, combined with freshwater run off during early Wisconsinan periods of warming probably facilitated dispersal for Arctic grayling between rivers by decreasing the salinity in the Beringian seas. Consequently, divergent groups of Arctic grayling likely expanded their ranges into drainages along the west coast of Alaska. Consequently, both mtDNA groups A and B are present in Seward Peninsula and the lower Yukon River (Figure 7) and there is tight clustering of these mtDNA lineages for the microsatellite data (Figure 10).

The disjunct distribution of mtDNA group B in North America (Figure 7, 8) may have resulted from dispersal into the south during the Wisconsinan glaciation. The fossil discovered in southern Alberta was dated between 22,000 and 50,000 years bp (before present) and is located in a region that remained ice free between the Cordilleran and Laurnetide ice sheets during the Wisconsinan (Burns 1991). It is conceivable that a corridor between the two ice masses existed between 50,000 and 20,000 bp and conditions may have been beneficial for cold adapted freshwater fish to colonize southern habitats. This is the best explanation for both the age of the fossils and genetic divergence estimates of lake trout (Wilson and Hebert 1998) and Arctic grayling mtDNA in this area (Figure 12) from their closest ancestors in the lower Mackenzie River (e.g. Arctic grayling in the Keel River, Table 5). Subsequent advance of the glaciers would have isolated these populations in the south between 17,000 and 10,000 ybp until the beginning of the Holocene when glacial Lake Agassiz permitted northward range expansion for Arctic grayling. It remains unclear why this north Beringian lineage was able to disperse south while the Nahanni group remained in the Mackenzie drainage.

This southward dispersal from north Beringia must have taken place at the same time or before genetic exchange between north and south Beringia because differentiation in microsatellite allele frequencies is apparent. Missouri origin Saskatchewan populations were consistently divergent from Beringian populations that contained mtDNA lineage B (Figure 10) and the largest proportion of microsatellite genetic variation occurred among regions when Saskatchewan populations were considered a distinct group (Table 8). Arctic grayling in Chukotka Peninsula in northeast Asia and the southeastern part of their range in North America (and possibly the lower Mackenzie River) may be all that remains of the pure north Beringian lineage. A massive lake existed in the Chuckchi Sea watershed (Lindsey and McPhail 1986) that may have had strong homogenizing influences on the north Beringian Arctic grayling (Skopets 1991), just as large proglacial lakes have had on other North American species (Lindsey and McPhail 1986, Pielou 1991, Dyke and Prest 1987).

Postglacial dispersal from Beringia and founding ofpopulations in previously glaciated regions

Among the two measures of diversity from microsatellite loci, the decline of mean number of alleles per locus with geographic distance from Beringia reflected postglacial range expansion more clearly than did expected heterozygosity (Figure 9). Likewise, haplotype diversity correlated more strongly with microsatellite diversity than nucleotide diversity (Figure 13), so was also more negatively correlated with geographic distance from Beringia. This was not surprising because isolated populations and regions do not receive migrants so the number of alleles and number of haplotypes can only increase through mutation. Once the drainage connections between watersheds diminished, there probably has not been sufficient time (10,000 to15,000 years) for mutation to be an important generator of genetic diversity in previously glaciated regions (mutation rate estimates are 10^{3} and 10^{6} per generation for microsatellites and mtDNA, respectively). Consequently, the number of alleles and the number of haplotypes within watersheds have increased negligibly since Arctic grayling expanded their range behind the receding glaciers. Expected heterozygosity can be high, however, in populations that have few alleles but two or more of them are common. Likewise, nucleotide diversity can be low in regions (and populations) that have many haplotypes, or high in regions that have few haplotypes, depending on the genetic relatedness among haplotypes. Consequently, the genetic signature of postglacial range expansion was most clearly reflected by genetic diversity that was not altered by the demographics within populations in previously glaciated regions (i.e. mean number of alleles per locus).

During postglacial dispersal, populations located adjacent to early Holocene dispersal corridors must have been primarily responsible for establishing colonies in previously glaciated regions (Hewitt 1999). Consequently, previously glaciated regions that show fixation or high frequency of haplotypes and alleles that were rare in the source refugia may reflect historical (and possibly current) population structure in the glacial refuge. This was most apparent in the disjunct southern mtDNA group B populations in Saskatchewan and Montana where unique alleles and haplotype 7 distinguished these populations from the putative source in north Beringia (Table 5, Figure 11 D). Common alleles in both regions suggest a north Beringian origin although these allele frequencies dramatically increased in the Saskatchewan grayling (likely due to founder events) that inflated the genetic distance estimates between the regions (Figures 10 A, 13; Hedrick 1999). There were not sufficient samples collected from Arctic Alaska to confirm that haplotype 7 and unique Saskatchewan alleles were absent from northern regions. Nevertheless, the simplest explanation for the geological evidence (fossils in Alberta) and divergence among mtDNA haplotypes (discussed above) is that the upper Missouri River was colonized by Arctic grayling from north Beringia during or just before the Wisconsinan glaciation.

Unique haplotypes in the upper Missouri River and Saskatchewan (Table 5) suggests long isolation from Beringia, and greater haplotype diversity in the upper Missouri River suggests postglacial dispersal took place from a Great Plains or upper Missouri refuge. Redenbach and Taylor (1999) discovered yet another group B haplotype (as well as haplotype 7) in the Madison River (upper Missouri drainage) that was not found in any samples in my study, suggesting that Arctic grayling survived glaciation near the upper Missouri River. Postglacial range expansion from a Great Plains or Missouri glacial refuge was facilitated for freshwater fish by the formation of glacial lakes (e.g. Glacial Lake Agassiz) that provided access into Arctic watersheds early in the Holocene (Dyke and Prest 1987). A more local distribution of the other group B haplotypes in the north (Table 5) suggests early Holocene range expansion from Beringia was restricted by geographic barriers near the Mackenzie delta (Lindsey and McPhail 1986).

Range expansion from a south Beringian glacial refuge

The decline of genetic diversity (mtDNA and microsatellite loci) within the south Beringian lineage from the lower Yukon to the Peace River (Figure 8, 9) suggests post-Wisconsinan range expansion took place from the Yukon River. Much of the diversity in the lower Yukon River, however, probably stems from genetic exchange with north Beringia (see Table 5). Nevertheless, range expansion into the upper Liard River watershed was most likely promoted by drainage connections between Finlayson Lake and Pelly River (Lindsey et al. 1981; Lindsey and McPhail 1986). The flow of Frances River (currently an upper Liard tributary) was blocked by an ice tongue about 10,000 years ago, forcing the flow northward into the Pelly/Yukon drainage and probably promoted colonization from the Yukon River into the upper Liard River and vise versa. Other opportunities for fauna exchange between the Liard River and Yukon River watersheds may also have been available further west at Teslin River headwaters.

The distribution of group A haplotypes suggests Arctic grayling from south Beringia expanded their range from more than one isolated region. Only haplotypes 1 and 3 were found in the Yukon drainage while the remaining group A haplotypes were found in previously glaciated regions across the continental divide in the Mackenzie River and Stikine River watersheds (Table 5, Figure 7). Haplotype 3 may have originated from downstream in the Yukon River in southwestern Beringia and haplotype 4 might have come from further inland, near the confluence between the Yukon and Pelly rivers. There is geological evidence that two cataclysmic volcanic eruptions occurred about 1,600 and 1,280 year ago that likely eliminated freshwater fish populations in the Teslin River and the lower Pelly River (Lindsey et al. 1981; Lindsey and McPhail 1986). The presence of haplotype 4 in the upper Liard River suggests it was once widely distributed in the upper Yukon River watershed prior to these events, but the upper Yukon River is now dominated by haplotype 3, suggesting that a different group of Arctic grayling expanded their range from the lower Yukon River. Divergent haplotypes of lake whitefish *(Coregonus clupeaformis)* are similarly distributed in the western part (Teslin River) of the upper Yukon River, also reflecting initial colonization by a Beringian lineage, followed by colonization from downstream by a widely distributed Eurasian lineage (Bernatchez et al. 1996). Sampling from a greater distribution of Arctic grayling in the Yukon River, particularly from regions that were not impacted by volcanic ash like the upper Pelly River and upper Teslin River, is needed to confirm this historical scenario for current population structure.

Absence of mtDNA lineages other than group A in the upper Liard River suggests that the geographic barrier at Hell Gate in the Liard Canyon is and was impassable to upstream dispersal by Arctic grayling. Interestingly group A haplotypes were found together with group C haplotypes in tributaries of the lower Liard River, and rare haplotypes were found either only in the lower Liard River (haplotype 6) or the lower part of the upper Liard River (haplotype 5, Table 5, Figure 7). Furthermore haplotype 4 was by far the most common and widely distributed group A haplotype in the lower Liard River and yet only half as frequent as haplotype 1 in the upper Liard River (Figure 7). These data suggest that there were multiple (at least two) colonizations of the upper Liard from the Yukon drainage: one from the Frances River (haplotypes 4, 5, 6) and the other via the Teslin River, which founded populations further upstream in the upper Liard as well as the upper Peace River.

From the Upper Liard drainage Arctic grayling probably used headwater connections created by receding glaciers to colonize the Stikine River and the Upper Peace River, perhaps via low elevation connections between the upper Turnagain River (Kechika/Liard) and the Stikine River (see Atagi and Haas 1996) and between the Kechika River and the Fox River (upper Peace). Low genetic diversity in the Peace River and Stikine River (Table 5, Figure 8) probably resulted from bottlenecks caused by marginal habitat in the headwater streams or short lived drainage connections. Extensive sampling in the Peace River (see Chapter 3) has confirmed a dramatic decline of diversity from the upper Liard River, but further sampling is needed in the Stikine River. There was a large lake that connected the upper Stikine with the upper Liard at Dease Lake (Lindsey and McPhail 1986) that may have permitted larger numbers (thus greater genetic diversity) of Arctic grayling to colonize the Stikine watershed.

Implications for Conservation of Arctic grayling

My study has identified three reciprocally monophyletic groups of Arctic grayling mtDNA associated with three distinct glacial refugia and has provided the first evidence for a Nahanni lineage for Arctic grayling in the Mackenzie watershed of North America. Genetic divergence among populations measured with nuclear loci was also associated with these mtDNA groups (Table 7, 8). These data also provided evidence for a fourth group, suggesting a north Beringian vicariant group survived the Wisconsinan glaciation near the upper Missouri River and subsequently founded populations in Saskatchewan (Table 8, Figure 8). This evidence for reciprocal monophyly of mtDNA and associated genetic divergence at nuclear loci suggests that at least three evolutionarily significant groups of Arctic grayling should be recognized in northwestern North America (Moritz 1994). These are located in the lower Liard/Mackenzie (Nahanni grayling), the Arctic coast including the mouth of the Mackenzie River, the Beaufort Sea and possibly Chukotka Peninsula (north Beringian grayling), and the northwest including the Yukon River, upper Liard River, Peace River and the west coast of British Columbia and Alaska (south Beringian grayling). A fourth disjunct group (Montana grayling) located in the southern and eastern extremes of the species range may also be considered unique although mtDNA divergence from north Beringia suggests time since isolation has not been high.

Molecular divergence measured at neutral loci is not necessarily a good predictor of adaptive differentiation between lineages or populations (Lynch 1996). Nevertheless, greater divergence suggests longer isolation; a history that increases the probability for adaptive differences to arise through chance mutation and then maintained by selection. Now that the locations of distinct lineages of Arctic grayling have been identified, further studies can be done in areas of secondary contact focused on investigating adaptive, morphological and life history differences. When in sympatry, adaptive differences between divergent lineages can become exaggerated if ecological conditions enable distinct ecotypes to persist in reproductive isolation (Bernatchez 1995; Schluter 1996; Taylor 1999; Bernatchez et al. 2000). Alternatively, introgression between divergent lineages might create unique populations by combining distinct adaptations. The distribution of mtDNA haplotypes has revealed regions of secondary contact are located in the lower Liard River (between Nahanni and south Beringian grayling) and Seward Peninsula and Yukon River (between north Beringian and south Beringian grayling). Secondary contact may also have taken place between Nahanni and north Beringian grayling in other regions in the Mackenzie drainage that were not extensively sampled in my study (e.g. further downstream and upstream from the Nahanni region). The geographic location of the Nahanni lineage suggests they have adapted to a different habitat from Beringian grayling, possibly more tolerant of turbid, low gradient streams and living in sympatry with a diverse freshwater fish fauna of Mississippi origin (McPhail and Lindsey 1970).

There are also likely to be adaptive differences between the two Beringian groups of *T. arcticus*. Postglacial dispersal across the continental divide into the Mackenzie watershed from the Yukon River early in the Holocene may have consisted of south Beringian grayling (Figure 8). If this is true, then Arctic grayling in the upper Peace River, upper Liard River and possibly the Pelly/Yukon River are a distinct group from Arctic and coastal Alaska. It would be interesting to investigate further the correlated distribution of scale size (McCart and Pepper 1972) with mtDNA group B.

Assuming that molecular diversity reflects genome wide genetic variation and that such diversity is important for long term persistence of populations under a wide range of environments (Frankham 1995), the north Beringian grayling may be the most resilient to environmental change. Given their wide geographic range and relatively recent connection with Siberia, they appear to have a superior ability to adapt and disperse into novel environments. The Great Plains populations located in Montana and Saskatchewan are the most recent offshoots from this source group. Relic populations in the Great Lakes (now extinct) and Montana suggest the range has contracted, probably due to their inability to adapt to a changing (warming) environment since the end of the Holocene. Low genetic diversity measures may signify especially sensitive populations, in which case populations located at the southern and eastern limit to the species range should be disturbed as little as possible. Evidence of bottlenecks in the lower Peace populations suggests they may be sensitive to environmental change due to low genetic variation. Alternatively, bottlenecking and associated low molecular variability has been associated with elevated levels of quantitative genetic variation of potential adaptive significance (Carson 1990; Armbrusteret al. 1998).

Evidence for gender biased gene flow among lineages is of particular interest to management of Arctic grayling. It is well known that Arctic grayling are highly sensitive to habitat alterations and once they have been extirpated from an environment attempts to re-establish populations have invariably been unsuccessful (Vincent 1950, Scott and Crossman 1973, Northcote 1995). Part of the reason for their inability to rebound from population decline may stem from strong spawning site fidelity; surrounding populations would tend not to recolonize neighboring regions. If females have strong spawning site fidelity but males tend to stray, then limited genetic differences between adjacent populations would be measurable even though they are demographically autonomous

(Avise 1995). An alternative explanation for the observed vivid geographic structure of mtDNA relative to nuclear markers could simply be stronger effects of genetic drift and founder events on smaller effective population size of mtDNA. Clearly homing behaviour in Arctic grayling needs to be studied further and will be addressed in the next chapter.

Table 1: Sample sites, sample sizes, haplotype diversity and nucleotide diversity of Arctic grayling mtDNA.

Population	Sample Size	Haplotype Diversity (SE)	Nucleotide Diversity(x100)
Beafort Sea Coast			
Sagavonirktok R.	17	0.5348 (0.0788)	0.0874
Seward Penninsula			
Niukluk R.	21	0.5110 (0.0185)	0.3543
Lower Yukon River			
Chena R.	20	0.5795 (0.0486)	0.1627
Upper Yukon River			
Teslin R.	15	0.4598 (0.0612)	0.0548
Plate L.	5	0.0000	0.0000
North Pacific Coast			
Stikine R.	10	0.0000	0.0000
Copper R	18	0.0000	0.0000
Becharof L.	10	0.0000	0.0000
Upper Liard River			
Franks L.	19	0.3983 (0.0692)	0.0942
Upper Tootsie L.	5	0.0000	0.0000
Turnagain R.	4	0.4286 (0.1687)	0.115
Trout R.	3	0.5333 (0.1721)	0.1533
Lower Liard River			
Muskwa R.	6	0.0000	0.0000
LaBiche R.	15	0.6805 (0.0476)	1.1047
Beaver R.	13	0.1477 (0.0888)	0.3141
Petitot R.	2	0.6667 (0.2041)	2.0418
Minnaker R	2	0.6667 (0.2041)	2.0418
MacKenzie River			
Keel R.	4	0.0000	0.0000
North Nahanni R.	4	0.0000	0.0000
Great Slave L.	16	0.1210 (0.0751)	0.0144
Upper Peace River			
Table R.	20	0.0000	0.0000
Anzac R.	20	0.0000	0.0000
Nation R.	20	0.0000	0.0000
Mesilinka R.	20	0.0974 (0.0621)	0.0114
Ingenika R.	20	0.0000	0.0000
Lower Peace River			
Burnt R.	24	0.1560 (0.0666)	0.1078
Beatton R	14	0.0000	0.0000
Saskatchewan			
Wollaston L.	20	0.0000	0.0000
Black L.	10	0.0000	0.0000
Upper Missouri River			
Maddison R.	2	0.0000	0.0000
Big Hole R.	1	0.0000	0.0000

Table 2: Sample sites, sample sizes and diversity measures (mean # of alleles and heterozygosity) from five microsatellite loci for all Arctic grayling samples. The diversity measures were derived from transformed allele frequency (see text) and sample sites are listed from the northwestern (Beringia at the top) to the southeastern (Saskatchewan) part of the species' range in North America (see Figure 8).

Population	Sample Size	Mean Number of Alleles per Locus (SE)	Microsatellite Locus									
			One8		Ots100		*BFRO04*		*Tar1*		*Tar8*	
			He	Ho	He	Ho	He	Ho	He	Ho	He	Ho
Beaufort Sea Coast												
Sagavonirktok River	20	2.6 (0.065)	0.305	0.300	0.000	0.000	0.499	0.550	0.570	0.450	0.474	0.316
Seward Penninsula												
Niukluk River	30	3.0 (0.043)	0.000	0.000	0.624	0.621	0.587	0.567	0.570	0.500	0.479	0.414
Yukon Drainage												
Koyukuk River	24	2.6 (0.054)	0.475	0.550	0.000	0.000	0.579	0.368*	0.679	0.684	0.090	0.095
Chena River	20	2.8 (0.065)	0.095	0.100	0.140	0.150	0.495	0.550	0.705	0.800	0.380	0.250*
Atlin L#1	10	1.2 (0.129)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.425	0.000
Atlin L#2	10	1.6 (0.129)	0.000	0.000	0.000	0.000	0.000	0.000	0.530	0.500	0.425	0.500
Teslin River	15	2.2 (0.086)	0.000	0.000	0.127	0.133	0.433	0.467	0.553	0.467	0.520	0.333
North Pacific Coast												
Becharof Lake	24	1.8 (0.054)	0.000	0.000	0.042	0.042	0.000	0.000	0.530	**0.435***	0.192	0.208
Copper River	24	2.4 (0.054)	0.042	0.042	0.121	0.125	0.358	0.250	0.617	0.500	no data	no data
Taku River	10	1.4 (0.129)	0.000	0.000	0.100	0.100	0.000	0.000	0.000	0.000	0.270	0.100
Stikine River	10	2.0 (0.129)	0.000	0.000	0.146	0.154	0.323	0.385	0.148	0.154	0.462	0.385
Upper Liard River												
Blue River	19	2.0 (0.068)	0.000	0.000	0.505	0.474	0.400	0.211	0.500	0.737	0.242	**0.053***
Lower Liard River												
Muskwa River	19	2.4 (0.068)	0.000	0.000	0.467	0.556	0.294	0.333	0.514	0.667	0.153	0.105
Beaver River	17	2.6 (0.076)	0.218	0.235	0.471	0.471	0.676	**0.353***	0.544	0.438	0.318	0.235
LaBiche River	18	2.2 (0.072)	0.056	0.056	0.283	0.333	0.539	0.500	0.594	0.611	0.000	0.000
Mackenzie River												
Great Slave Lake	16	2.8 (0.081)	0.269	0.188	0.275	0.019	0.331	0.375	0.814	0.714	0.175	0.188
Upper Peace River												
Table River	60	1.4 (0.022)	0.000	0.000	0.436	0.467	0.000	0.000	0.485	0.518	0.000	0.000
Anzac River	60	1.6 (0.022)	0.000	0.000	0.395	0.400	0.017	0.017	0.542	0.517	0.000	0.000
Nation River	49	1.4 (0.026)	0.000	0.000	0.506	0.471	0.000	0.000	0.497	0.441	0.000	0.000
Mesilinka River	36	1.6 (0.036)	0.000	0.000	0.357	0.457	0.029	0.029	0.649	0.657	0.000	0.000
Ingenika River	23	1.6 (0.056)	0.000	0.000	0.085	0.087	0.230	0.261	0.661	0.696	0.000	0.000
Finlay River	29	1.4 (0.046)	0.000	0.000	0.000	0.000	0.070	0.071	0.459	0.483	0.000	0.000
Lower Peace River												
Burnt River	34	1.6 (0.038)	0.000	0.000	0.503	0.500	0.167	0.182	0.058	0.059	0.000	0.000
Halfway River	18	1.8 (0.072)	0.000	0.000	0.200	0.222	0.539	0.667	0.000	0.000	0.000	0.000
Beatton River	14	1.6 (0.092)	0.000	0.000	0.000	0.000	0.520	0.615	0.443	0.429	0.000	0.000
Saskatchewan												
Wollaston Lake	20	1.4 (0.065)	0.500	0.450	0.000	0.000	0.360	**0.150***	0.500	0.450	0.000	0.000
Black Lake	20	2.0 (0.065)	0.447	0.474	0.000	0.000	0.530	0.400	0.295	0.250	nodata	no data

- **significant heterozygote deficiencies ($p \leq 0.05$) are marked by an asterisk**

Table 3: List of primer sequences, references and PCR annealing temperatures (hot/cold) used on the five microsatellite loci found variable in Arctic grayling.

Locus	Source Species	Reference	Annealing Temperature (OC)	N	Total # of Alleles	Alleles Size Range (bp)
One 8	Sockeye Salmon	Scribner et al. 1996	62/60	625	10	167-187
*Ots*100	Chinook Salmon	Nelson and Beacham 1999	60/58	629	3	220-300
BFRO 4	European grayling	Snoj et al. 1999	63/61	624	5	162-172
*Tar 1**	Arctic grayling	F ACA TAT CAT TCT TAG CAT ATC R CAA AAT AGT AAT TGA AAT GC	52/50	622	7	79-110
Tar 8*	Arctic grayling	F GAA GTG GTG GAT GTG AAT TT R GGC ATG CAT TTT TAC CTC TA	56/54	621	7	184-200

* E.B. Taylor unpublished data

Table 4: List of restriction enzymes, recognition sequence (base pairs) and site matrix for each mtDNA fragment pattern found.

Restriction Enzyme	Recognition Site	Fragment Pattern	Site Matrix
Alu I	AGCT	A	111111111101[1]
		B	111111111111[1]
Ban I	GGPyPuCC	A	1111
Dpn II	GATC	A	00111111
		B	10111111
		C	01111111
Hae III	GGCC	A	11111111111
		B	01111111111
		C	11110110111
Hinf I	GANTC	A	11111101011
		B	01111101011
		C	11111101111
		D	01111100011
		E	11111100011
Rsa I	GTAC	A	1111111111
		B	1101111111
		C	1101110111
Sty I	CC(AA/TT)GG	A	1111
		B	1110
Taq I	TCGA	A	1111111

Restriction Enzyme	Recognition Site	Fragment Pattern	Site Matrix
Ava II	GG(A/T)CC	A	101
		B	111
Bfa I	CTAG	A	111111111
BstUI	CGCG	A	00010
		B	11010
		C	10101
DdeI	CTNAG	A	0011111101101
		B	0011111101111
		C	0111111101101
		D	1011111101101
		E	0011111111101
Hha I	GCGC	A	101111
		B	111111
		C	111011
Hinc II	GTPyPuAC	A	11111
MspI	CCGG	A	111111111

Table 5: Composite haplotypes of Arctic grayling mitochondrial DNA showing the frequency of each haplotype within sampled populations.

Haplotype Number	Fragment Pattern	Alaska and Yukon							British Columbia						
		Beaufort Sea Coast	Seward Peninsula	Bristol Bay	Pacific Coast	Lower Yukon River	Upper Yukon River		Pacific Coast	Upper Liard River				PeaceRiver	
		Sagavonirktok River	Niukluk River	Becharof Lake	Copper River	Chena River	Teslin River	Plate Lake	Stikine River	Blue River	Tumagain River	Tootsie Lake	Trout River	Upper Peace River	Lower Peace River
Haplotype 1	AAAAAAAAAAAAAAA		10*	10*	18*	10	15			19	1	5		99	36
Haplotype 2	AAAAAAAAAAABAAA													1	
Haplotype 3	AAAABAAAAAAAAAA					7		5							
Haplotype 4	AAABBAAAAAAAAAA								10		3		2		
Haplotype 5	AACABAAAAAAAAAA												1		
Haplotype 6	AAABCAAAAAAAAAA														
Haplotype 7	BAAAABAAAABCBAA														2
Haplotype 8	BAAAABAAAABABAA	3				2									
Haplotype 9	BAAAABAAAABEBAA	11	11*												
Haplotype 10	BAAAAAAAAABABAA	3													
Haplotype 11	BABCDCBABACDCAA														
Haplotype 12	BABCECBABACDCAA														

Haplotype Number	Fragment Pattern	British Columbia					Northwest Territories			Saskatchewan		Montana	
		Lower Liard River					Mackenzie River			Upstream of Athabaska Lake		Upper Missouri River	
		Muskwa River	Beaver River	LaBiche River	Minnaker River	Petitot River	Keel	North Nahanni	Great Slave Lake	Black Lake	Wollaston Lake	Maddison River	Big Hole River
Haplotype 1	AAAAAAAAAAAAAAA			2									
Haplotype 2	AAAAAAAAAAABAAA												
Haplotype 3	AAAABAAAAAAAAAA												
Haplotype 4	AAABBAAAAAAAAAA		1	5	1								
Haplotype 5	AACABAAAAAAAAAA												
Haplotype 6	AAABCAAAAAAAAAA												
Haplotype 7	BAAAABAAAABCBAA									10	20	2	1
Haplotype 8	BAAAABAAAABABAA						4						
Haplotype 9	BAAAABAAAABEBAA												
Haplotype 10	BAAAAAAAAABABAA												
Haplotype 11	BABCDCBABACDCAA	6	12	5	1	2		4	15				
Haplotype 12	BABCECBABACDCAA								1				

* = populations were only analysed with diagnostic enzymes, see text for details

Table 6: Average percent nucleotide divergence (Nei and Miller 1990) among and within haplotype groups. Range of values are in parenthises (no range for group C as there were only two haplotypes and one divergence measure)

	Group A	Group B	Group C
Group A	0.245 (0.113-0.459)		
Group B	0.746 (0.450-0.916)	0.166 (0.110-0.223)	
Group C	2.060 (1.886-2.271)	1.649 (1.498-1.745)	0.115

Table 7: Analysis of mtDNA variance (AMOVA) among populations grouped into geographic hierarchies on either side of the Rocky Mountain divide (East vs West) and putative origins from glacial refugia (NB = North Beringia, SB = South Beringia, N = Nahanni, NB *(vicar)* = Saskatchewan and Montana populations).

Geographic Hierarchy	Variance Component	F Statistic	Percent Variation	*P*
NB vs SB vs N	Among Regions	0.81	80.82	0.000
	Among Populations	0.52	10.02	0.000
	Within Populations among groups	0.91	9.17	0.000
NB vs SB vs N vs NB(vicar.)	Among Regions	0.78	77.8	0.000
	Among Populations	0.47	10.34	0.000
	Within Populations among groups	0.88	11.8	0.000
West vs East	Among Regions	0.34	34.45	0.001
	Among Populations	0.81	53.33	0.000
	Within Populations among groups	0.87	12.2	0.001

Table 8: Analysis of nuclear DNA (microsatellite) variance among populations grouped into geographic hierarchies on either side of the continental divide (East vs West) and putative origins from glacial refugia (NB = north Beringia, SB = south Beringia, N = Nahanni, NB *(vicar)* = Saskatchewan populations).

Geographic Hierarchy	Variance Component	F Statistic	Percent Variation	*P*
NB vs SB vs N	Among Regions	0.24	24.3	0.000
	Among Populations	0.33	24.7	0.000
	Within Populations among groups	0.49	51.0	0.000
NB vs SB vs N vs NB *(vicar.)*	Among Regions	0.32	32.1	0.000
	Among Populations	0.28	19.3	0.000
	Within Populations among groups	0.51	48.6	0.000
West vs East	Among Regions	0.18	18.3	0.000
	Among Populations	0.35	28.7	0.000
	Within Populations among groups	0.47	53.0	0.000

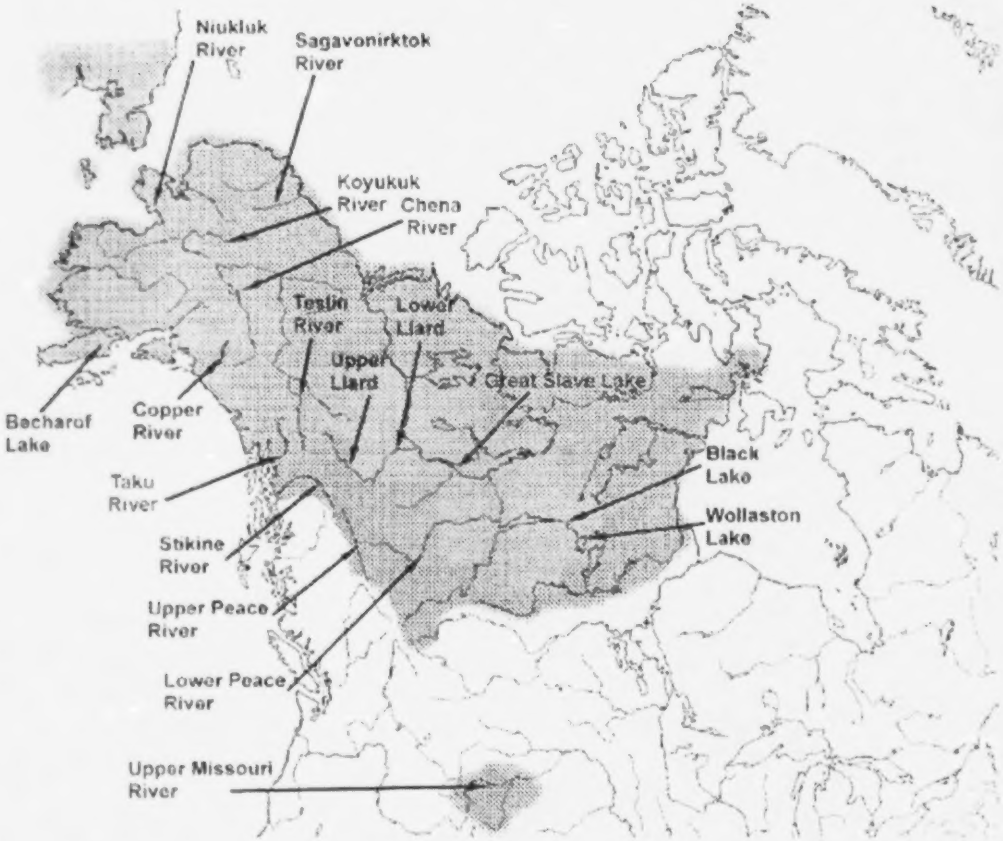

Niukluk River
Sagavonirktok River
Koyukuk River
Chena River
Teslin River
Lower Liard
Upper Liard
Great Slave Lake
Becharof Lake
Copper River
Black Lake
Taku River
Wollaston Lake
Stikine River
Upper Peace River
Lower Peace River
Upper Missouri River

Figure 1: Geographic distribution of Arctic grayling sample sites. Shaded area shows the distribution of *T. acticus* in North America and a small portion of Eastern Siberia (Scott and Crossman 1973).

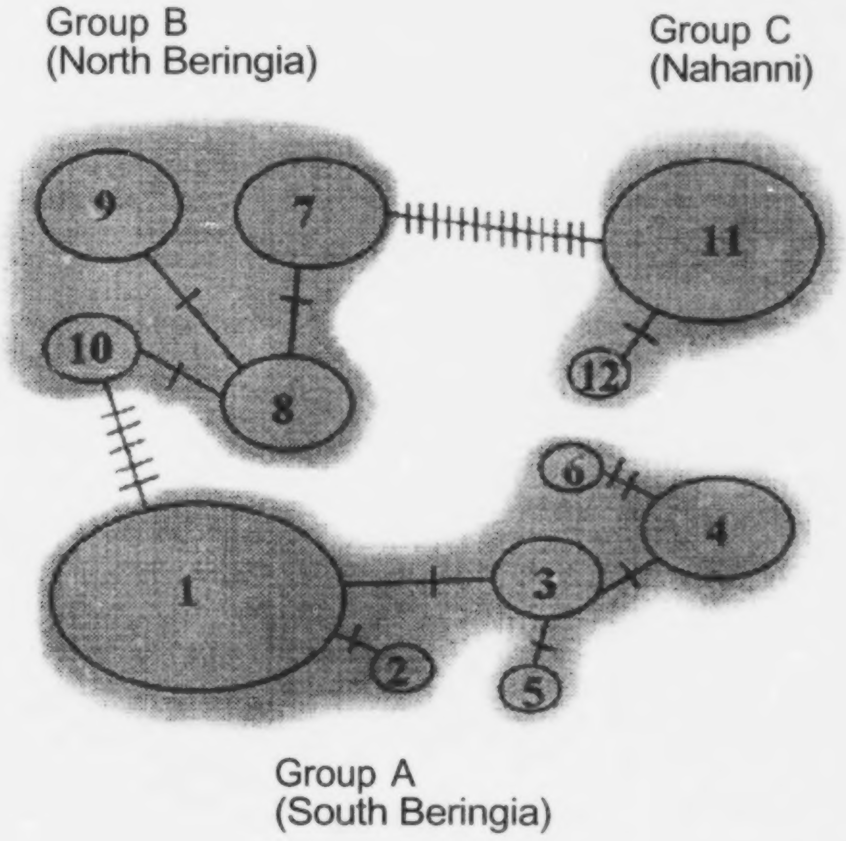

Group B
(North Beringia)
Group C
(Nahanni)
9
7
11
10
8
12
6
4
1
3
2
5
Group A
(South Beringia)

Figure 2: Parsimony network showing mutational relationships among the twelve Arctic grayling RFLP haplotypes (numbers inside ovals). The size of the ovals reflects the relative frequency of that haplotype within the data set. Each slash represents a loss or gain of a restriction site.

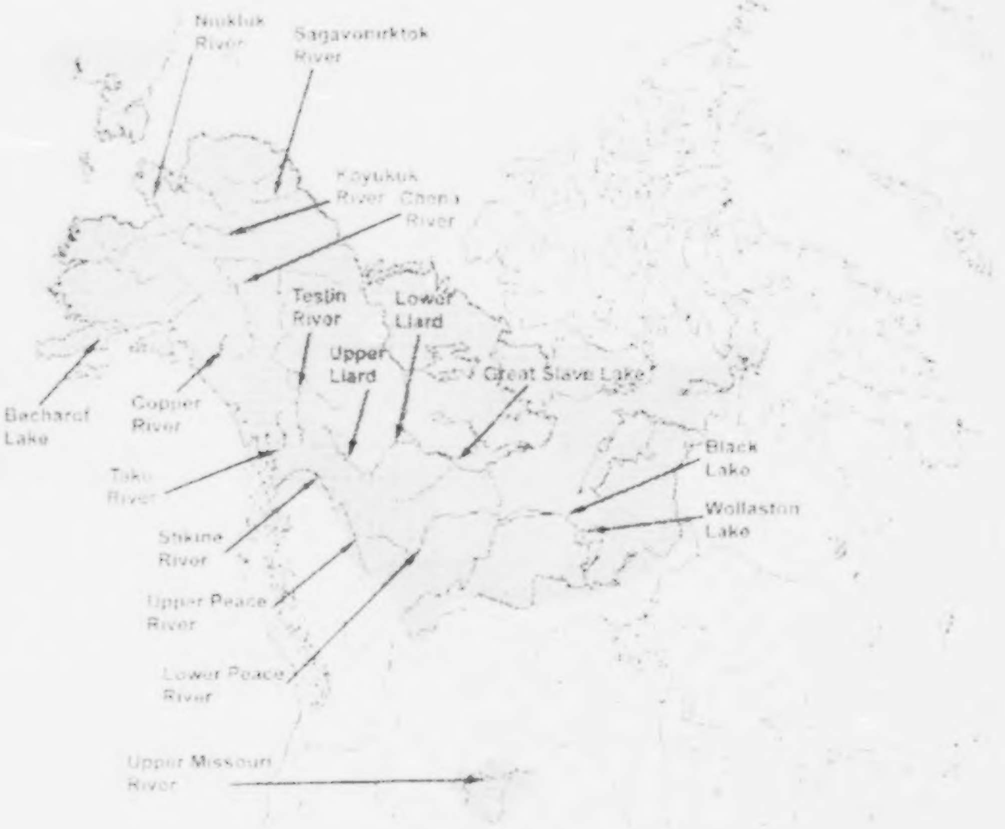

Niukluk River
Sagavanirktok River
Koyukuk River
Chena River
Teslin River
Lower Liard
Upper Liard
Great Slave Lake
Becharof Lake
Copper River
Black Lake
Taku River
Wollaston Lake
Stikine River
Upper Peace River
Lower Peace River
Upper Missouri River

Figure 1: **Geographic distribution of Arctic grayling sample sites. Shaded area shows the distribution of *T. acticus* in North America and a small portion of Eastern Siberia (Scott and Crossman 1973).**

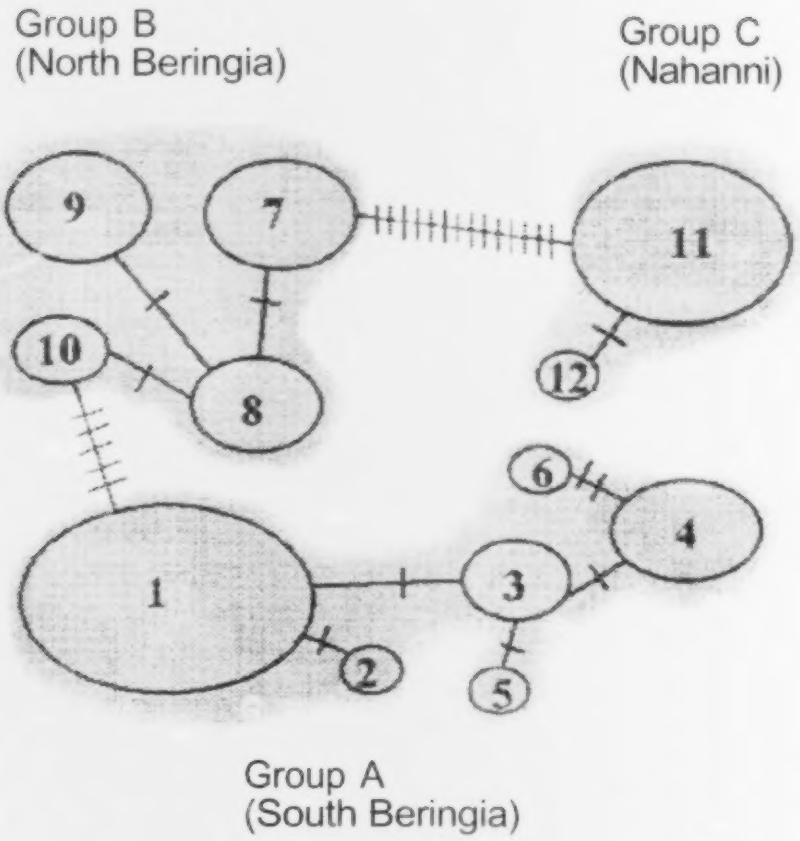

Group B
(North Beringia)
Group C
(Nahanni)
9
7
11
10
8
12
6
4
1
3
2
5
Group A
(South Beringia)

Figure 2: Parsimony network showing mutational relationships among the twelve Arctic grayling RFLP haplotypes (numbers inside ovals). The size of the ovals reflects the relative frequency of that haplotype within the data set. Each slash represents a loss or gain of a restriction site.

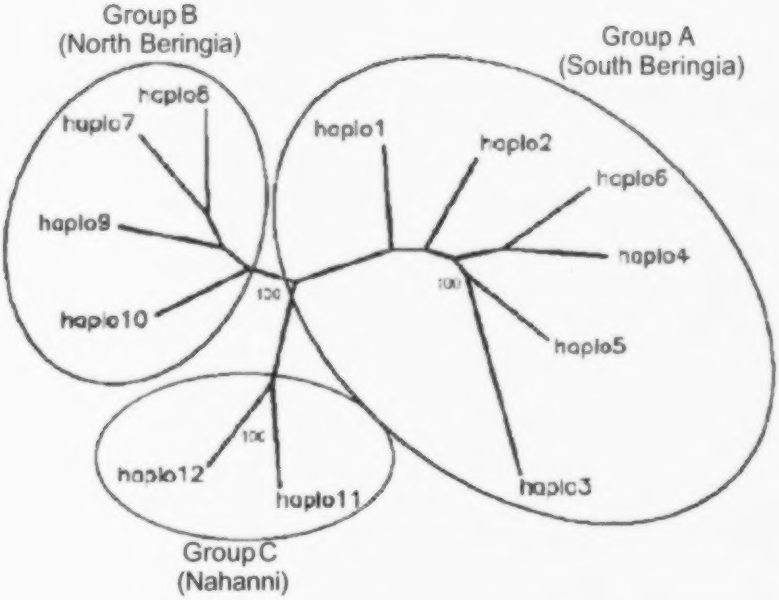

Group B
(North Beringia)
Group A
(South Beringia)
haplo8
haplo7
haplo9
haplo10
haplo1
haplo2
haplo6
haplo4
haplo5
haplo3
haplo12
haplo11
100
100
100
Group C
(Nahanni)

Figure 3: Consensus tree from Wagner parsimony analysis of the site matrix generated from twelve mtDNA RFLP Arctic grayling haplotypes. The numbers at the branch forks are the proportion from 1000 bootstrap replicates that the haplotypes at the branch tips occurred among the trees. Only bootstrap values greater than 50% are shown.

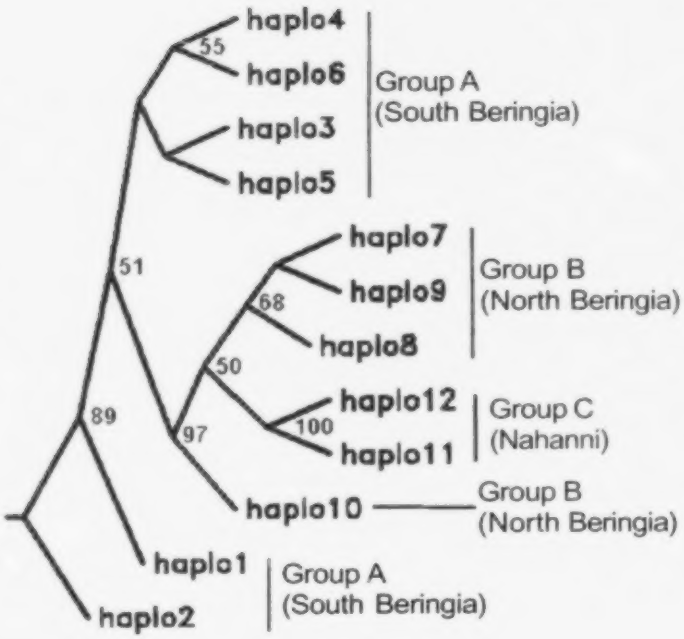

haplo4
55
haplo6
haplo3
haplo5
Group A
(South Beringia)
haplo7
haplo9
haplo8
Group B
(North Beringia)
51
68
50
haplo12
haplo11
100
Group C
(Nahanni)
89
97
haplo10
Group B
(North Beringia)
haplo1
haplo2
Group A
(South Beringia)

Figure 4: **Neighbor-Joining consensus cladogram of pairwise nucleotide divergence estimates (Nei's d) among twelve mtDNA RFLP haplotypes of Arctic grayling. Values at the branch forks are the proportion (%) from 1000 bootstrap replicates that the haplotypes at the branch tips occurred among the trees. Only bootstrap values greater than 50% are shown. Branch lengths are not to scale.**

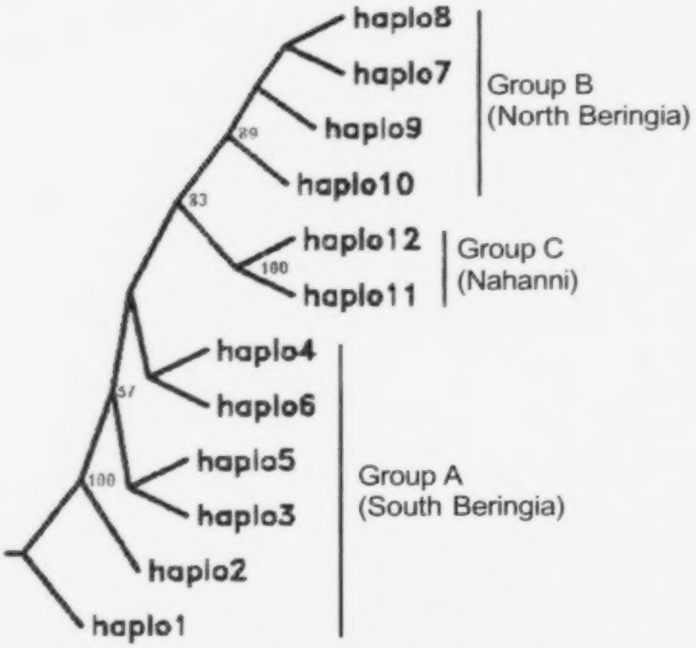

haplo8
haplo7
haplo9
haplo10
haplo12
haplo11
haplo4
haplo6
haplo5
haplo3
haplo2
haplo1
89
83
100
57
100
Group B
(North Beringia)
Group C
(Nahanni)
Group A
(South Beringia)

Figure 5: **UPGMA consensus cladogram of pairwise nucleotide divergence estimates (Nei's *d*) among twelve RFLP haplotypes of Arctic grayling. Values at the branch forks are the proportion (%) from 1000 bootstrap replicates that the haplotypes at the branch tips occurred among the trees. Only bootstrap values greater than 50% are shown. Branch lengths are not to scale.**

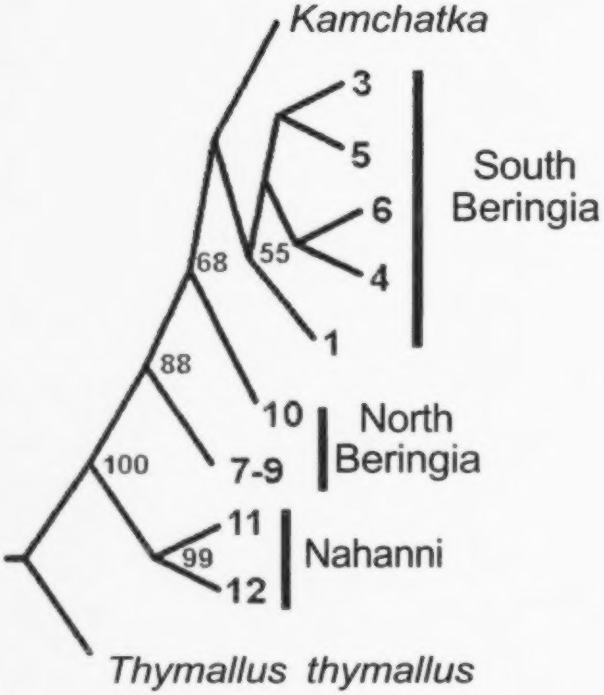

Kamchatka
3
5
6
4
1
South Beringia
68
55
88
10
7-9
North Beringia
100
11
99
12
Nahanni
Thymallus thymallus

Figure 6: Consensus tree from Wagner parsimony showing the evolutionary relationships among the three haplotype groups (North Beringian, South Beringian and Nahanni) relative to Arctic grayling from the Kamchatkan Peninsula in Siberia, and rooted with a European grayling *(Thymallus thymallus)* haplotype. The haplotypes (numbers correspond with haplotypes in Figure 2) in this tree were determined using the same eight restrictions enzymes used by Redenbach and Taylor (1999). Numbers at the branch fork show the percentage of times out of 1000 bootstrap replicates the haplotypes at the branch tips occurred among the trees.

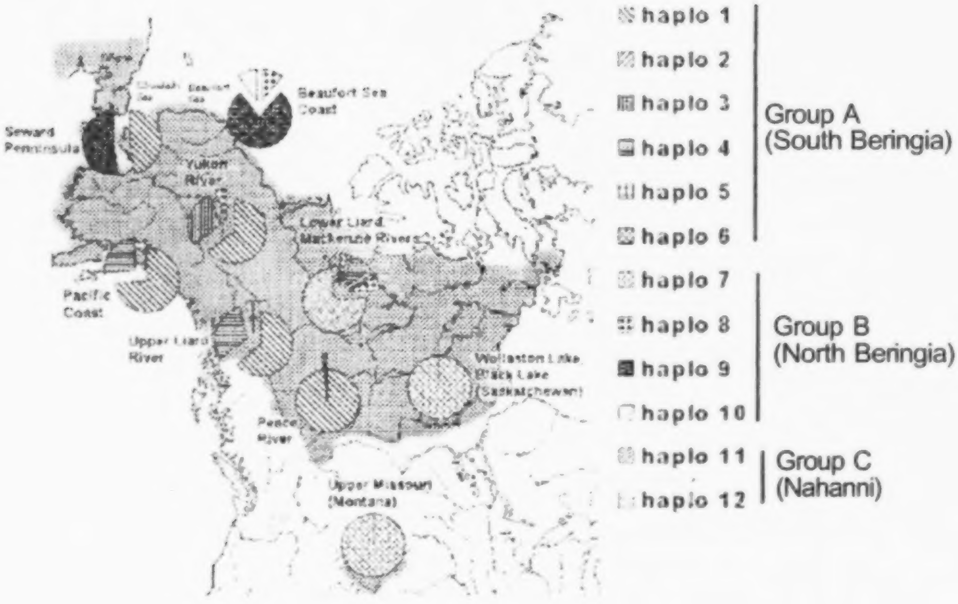

Seward Peninsula
Beaufort Sea Coast
Yukon River
Lower Liard Mackenzie Rivers
Pacific Coast
Upper Liard River
Wollaston Lake Black Lake (Saskatchewan)
Peace River
Upper Missouri (Montana)
haplo 1
haplo 2
haplo 3
haplo 4
haplo 5
haplo 6
haplo 7
haplo 8
haplo 9
haplo 10
haplo 11
haplo 12
Group A (South Beringia)
Group B (North Beringia)
Group C (Nahanni)

Figure 7: Distribution and relative abundance of haplotypes and haplotype groups throughout the range of Arctic grayling in North America. Shaded area shows the current distribution of the species in North America and and includes the north-eastern tip of Siberia.

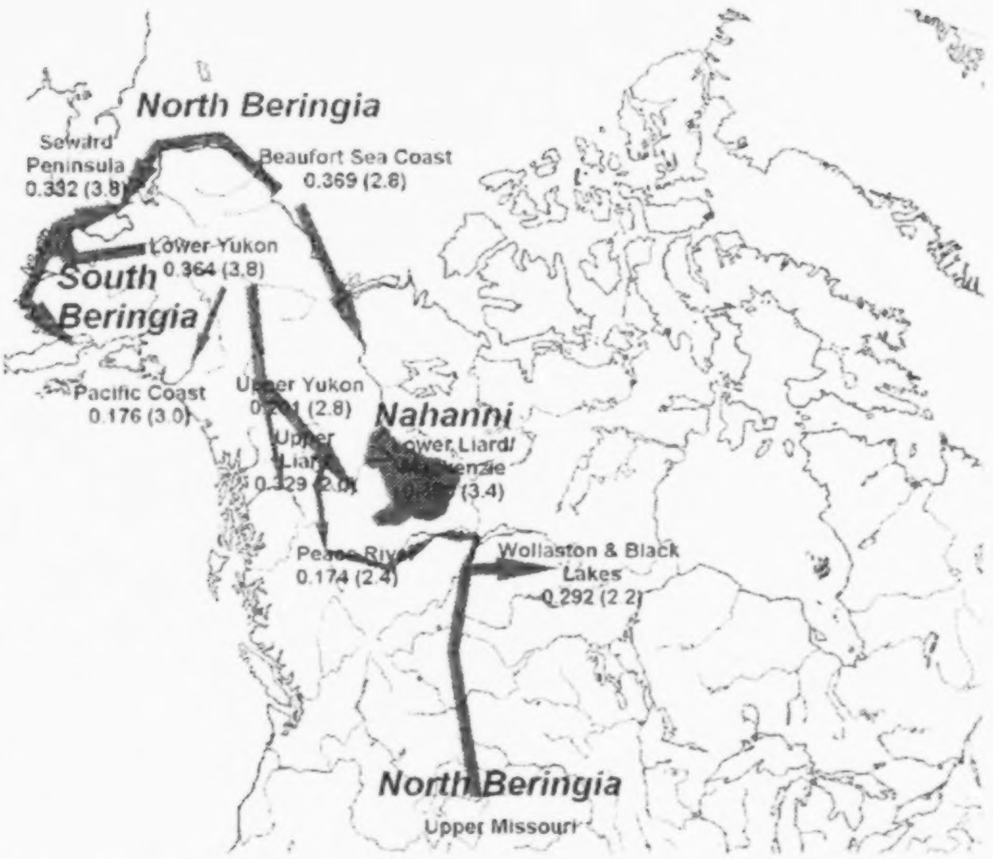

North Beringia
Seward Peninsula
0.332 (3.8)
Beaufort Sea Coast
0.369 (2.8)
Lower Yukon
0.364 (3.8)
South Beringia
Pacific Coast
0.176 (3.0)
Upper Yukon
(2.8)
Nahanni
Lower Liard/
Mackenzie
(3.4)
Upper Liard
0.329 (2.0)
Peace River
0.174 (2.4)
Wollaston & Black Lakes
0.292 (2.2)
North Beringia
Upper Missouri

Figure 8: Geographic distribution of genetic diversity at microsatellite loci. Both expected heterozygosity and mean number of alleles per locus (in parentheses) are shown. Arrows show inferred postglacial dispersal routes from glacial refugia, labelled with large font (see Figure 7). Shaded area shows the known distribution of the Nahanni mtDNA lineage.

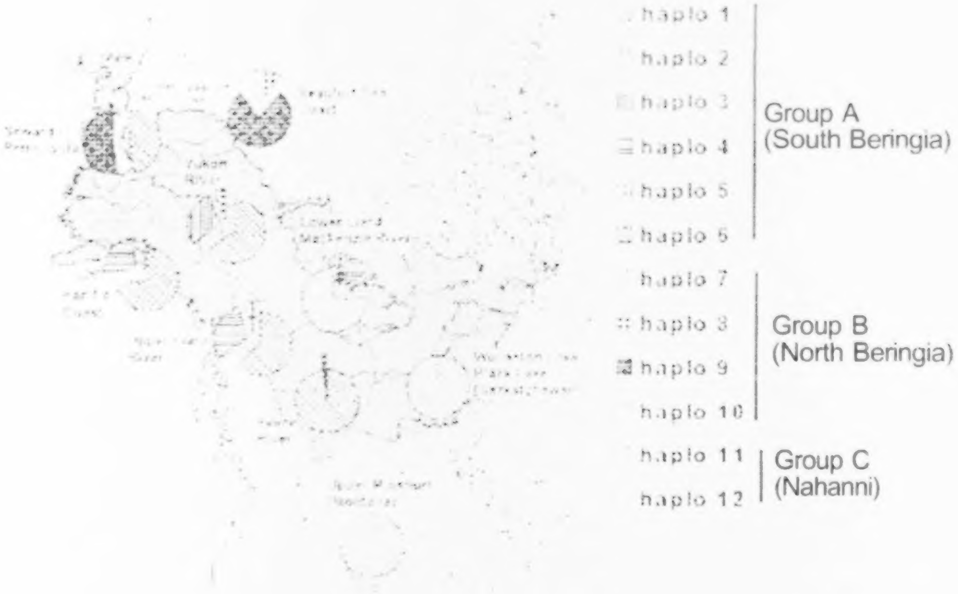

Seward Peninsula
Yukon River
haplo 1
haplo 2
haplo 3
haplo 4
haplo 5
haplo 6
haplo 7
haplo 8
haplo 9
haplo 10
haplo 11
haplo 12
Group A (South Beringia)
Group B (North Beringia)
Group C (Nahanni)

Figure 7: Distribution and relative abundance of haplotypes and haplotype groups throughout the range of Arctic grayling in North America. Shaded area shows the current distribution of the species in North America and and includes the north-eastern tip of Siberia.

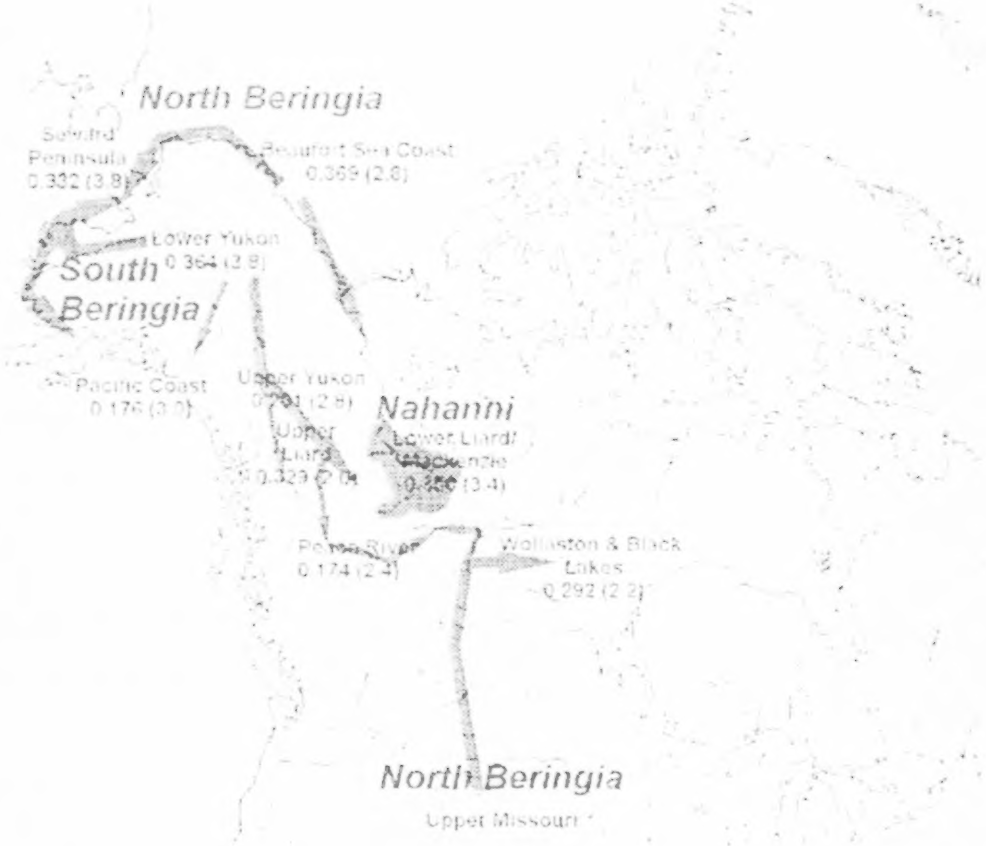
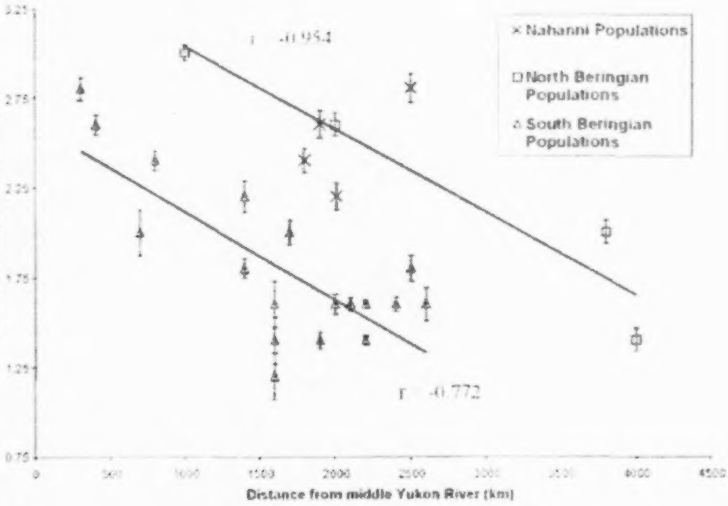

North Beringia
Seward Peninsula
0.332 (3.8)
Beaufort Sea Coast
0.369 (2.8)
Lower Yukon
0.364 (2.8)
South Beringia
Pacific Coast
0.176 (3.0)
Upper Yukon
(2.8)
Nahanni
Upper Liard
0.329 (2.0)
Lower Liard/ Mackenzie
(3.4)
River
0.174 (2.4)
Wollaston & Black Lakes
0.292 (2.2)
North Beringia
Upper Missouri

Figure 8: Geographic distribution of genetic diversity at microsatellite loci. Both expected heterozygosity and mean number of alleles per locus (in parentheses) are shown. Arrows show inferred postglacial dispersal routes from glacial refugia, labelled with large font (see Figure 7). Shaded area shows the known distribution of the Nahanni mtDNA lineage.

A: Mean number of alleles per locus versus geographic distance (km).



B: Expected heterozygosity versus geographic distance (km).

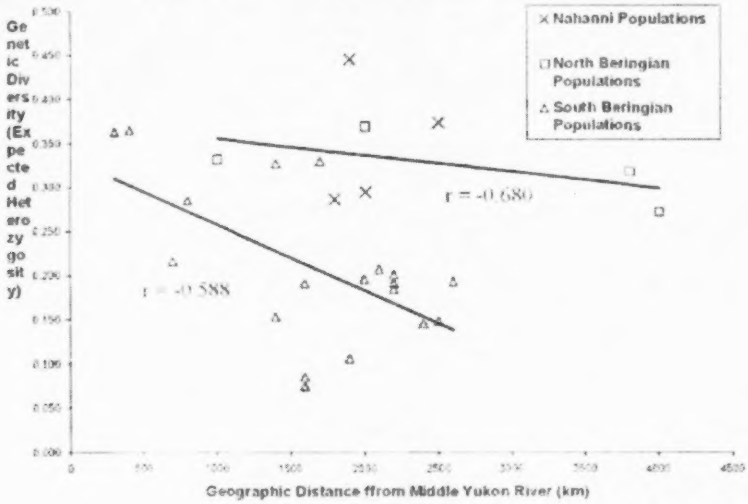
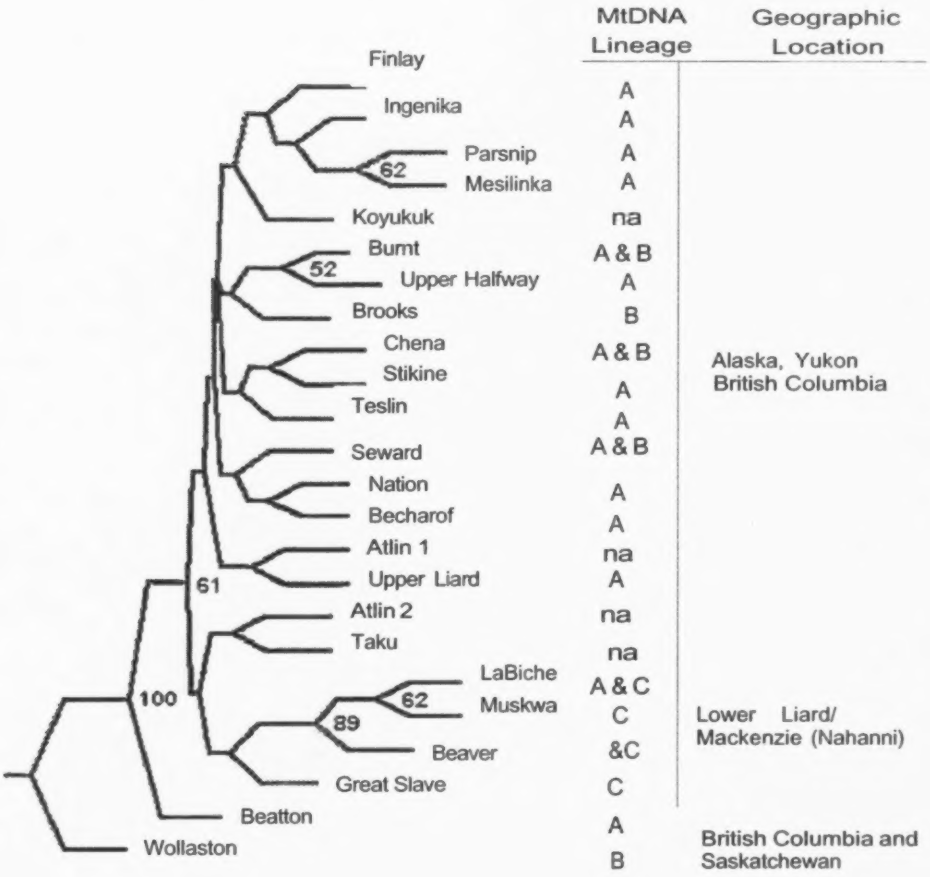


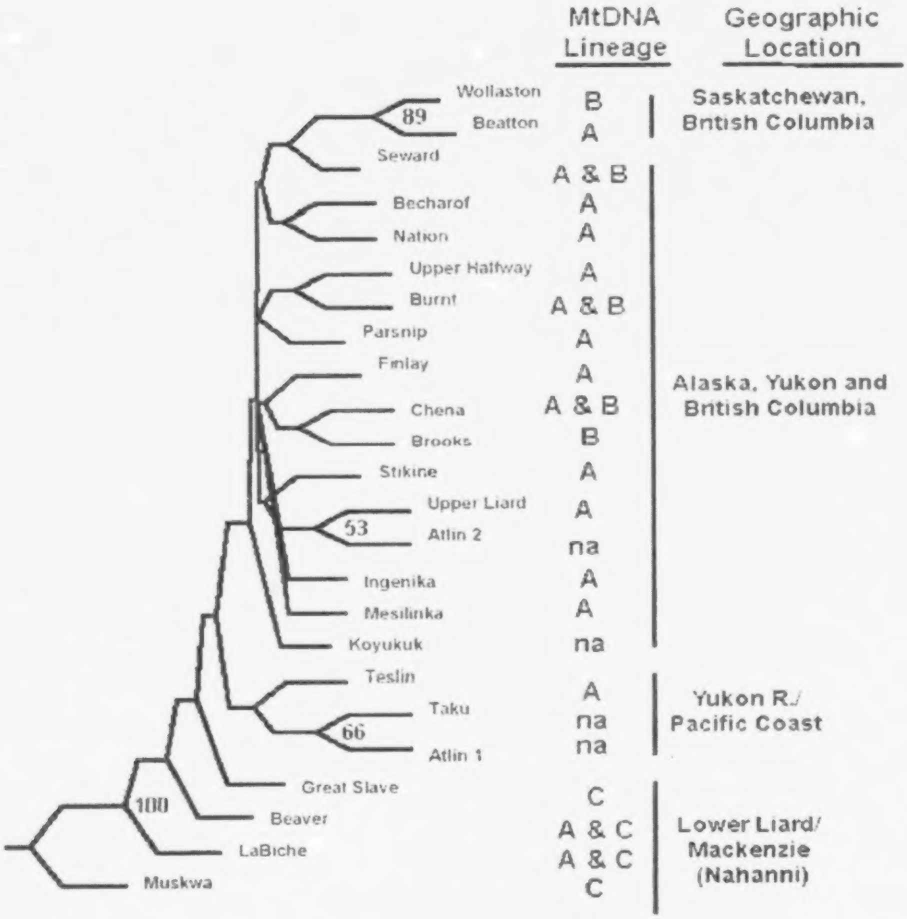
Figure 9: Change in microsatellite genetic diversity within populations versus their geographic distance from the middle Yukon River, at the confluence with the Koyukuk River (see Figure 1). The three population groups (Nahanni, north Beringia and south Beringia) were designated from the mtDNA lineage they contain (see Figure 7). Regression lines (for illustration) and r values are shown only for north and south Beringian groups.

Figure 10: Cluster analysis of genetic distance estimates (C-S Chord Distance and Nei's original distance) from microsatellite allele frequencies among populations. The mitochondrial DNA lineage that is known for each population and the geographic location of the populations within each group are also shown. For all of the trees, numbers at the branch forks are the percentage that the populations at the branch tips to the right of the fork occurred among the trees from 100 bootstrap replicates. Branch lengths are not to scale.

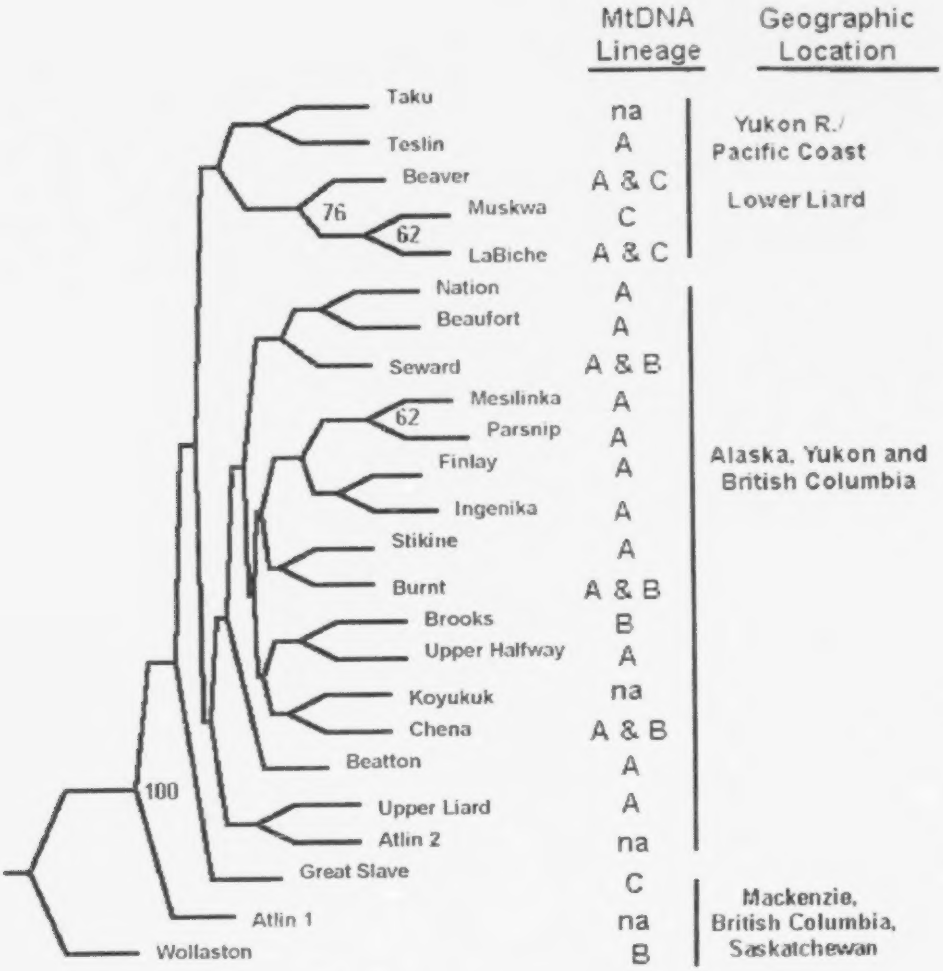
A: UPGMA consensus cladogram of C-S chord distance estimates.


MtDNA Lineage
Geographic Location
Finlay
Ingenika
Parsnip
62
Mesilinka
Koyukuk
Burnt
52
Upper Halfway
Brooks
Chena
Stikine
Teslin
Seward
Nation
Becharof
Atlin 1
Upper Liard
61
Atlin 2
Taku
LaBiche
62
Muskwa
89
Beaver
100
Great Slave
Beatton
Wollaston
A
A
A
A
na
A & B
A
B
A & B
A
A
A & B
A
A
na
A
na
na
A & C
C
&C
C
A
B
Alaska, Yukon British Columbia
Lower Liard/ Mackenzie (Nahanni)
British Columbia and Saskatchewan

B: N-J consensus cladogram of C-S chord distance estimates.


MtDNA Lineage
Geographic Location
Wollaston B
Beatton A
89
Saskatchewan, British Columbia
Seward A & B
Becharof A
Nation A
Upper Halfway A
Burnt A & B
Parsnip A
Finlay A
Chena A & B
Brooks B
Stikine A
Upper Liard A
53
Atlin 2 na
Ingenika A
Mesilinka A
Koyukuk na
Alaska, Yukon and British Columbia
Teslin A
Taku na
66
Atlin 1 na
Yukon R./ Pacific Coast
Great Slave C
Beaver A & C
LaBiche A & C
Muskwa C
100
Lower Liard/ Mackenzie (Nahanni)

C: UPGMA consensus cladogram of Nei's genetic distance estimates.


MtDNA Lineage
Geographic Location
Taku na
Teslin A
Beaver A & C
76
Muskwa C
62
LaBiche A & C
Yukon R./ Pacific Coast
Lower Liard
Nation A
Beaufort A
Seward A & B
Mesilinka A
62
Parsnip A
Finlay A
Ingenika A
Stikine A
Burnt A & B
Brooks B
Upper Halfway A
Koyukuk na
Chena A & B
Beatton A
100
Upper Liard A
Atlin 2 na
Alaska, Yukon and British Columbia
Great Slave C
Atlin 1 na
Wollaston B
Mackenzie, British Columbia, Saskatchewan

D: N-J cladogram of Nei's genetic distance estimates.

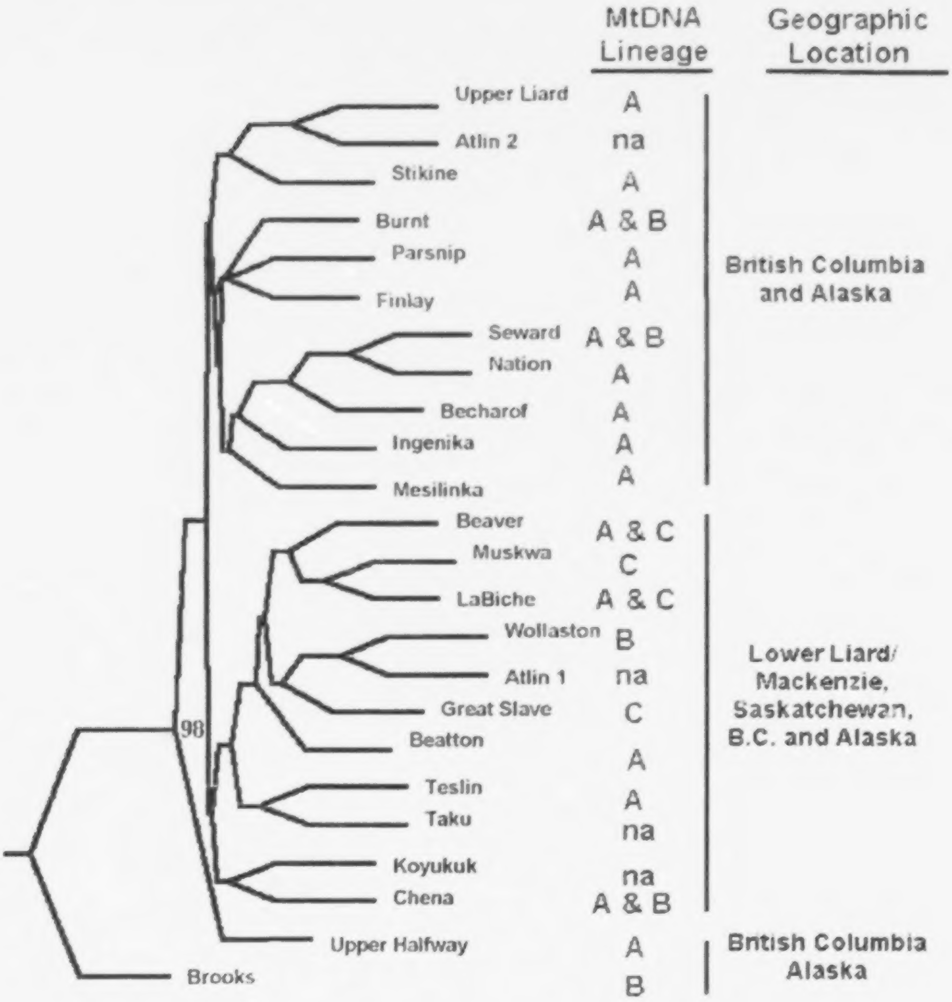
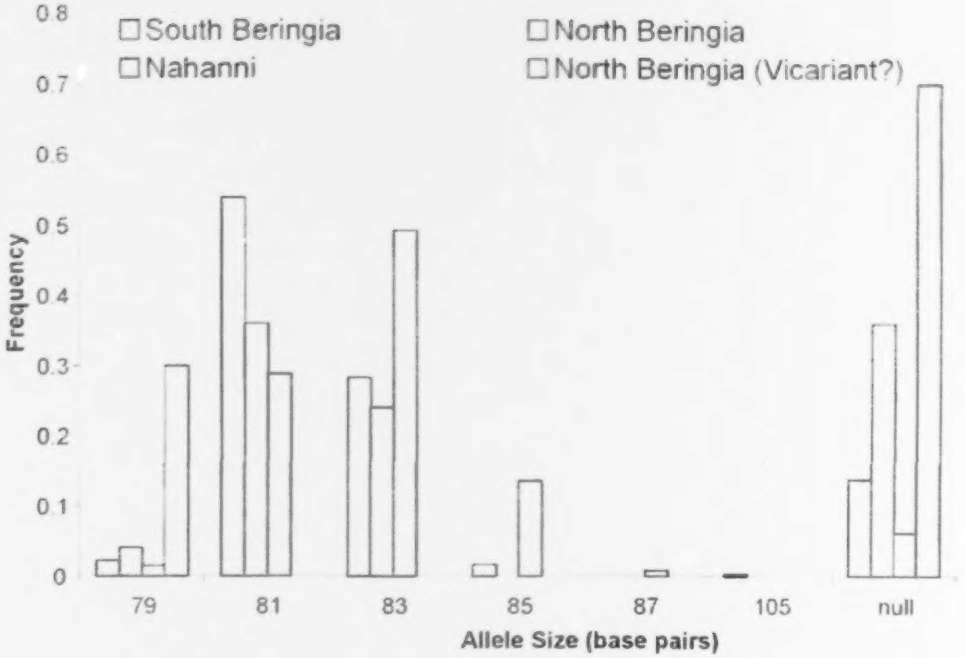
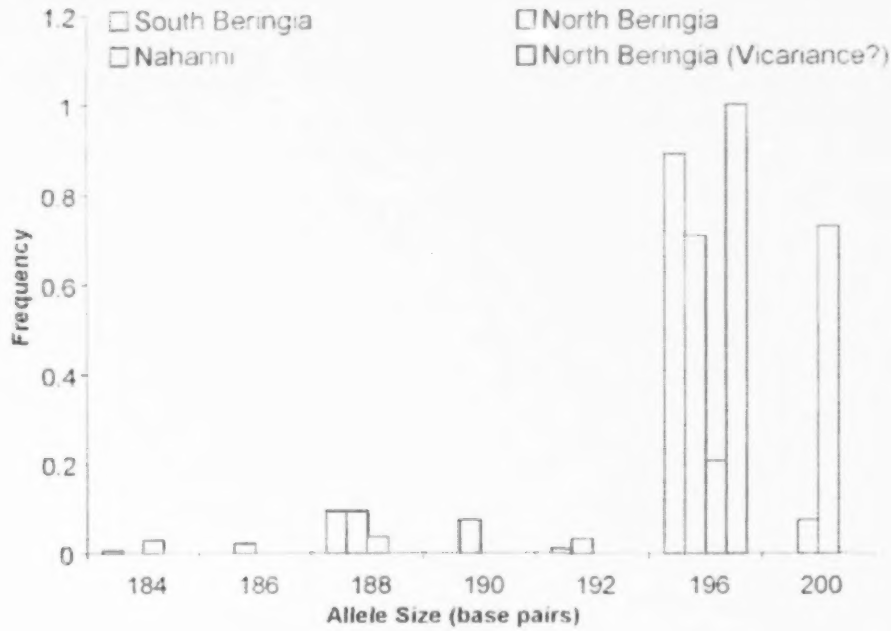

MtDNA Lineage
Geographic Location
Upper Liard A
Atlin 2 na
Stikine A
Burnt A & B
Parsnip A
Finlay A
Seward A & B
Nation A
Becharof A
Ingenika A
Mesilinka A
Beaver A & C
Muskwa C
LaBiche A & C
Wollaston B
Atlin 1 na
Great Slave C
Beatton A
Teslin A
Taku na
Koyukuk na
Chena A & B
Upper Halfway A
Brooks B
98
British Columbia and Alaska
Lower Liard/ Mackenzie, Saskatchewan, B.C. and Alaska
British Columbia
Alaska

Figure 11: Allele frequencies at microsatellite loci found among populations groups. The populations were grouped according to the predominant mtDNA lineage they contained (see Figure 7). North Beringia (vicariant) group consists of Saskatchewan populations and the North Beringia group

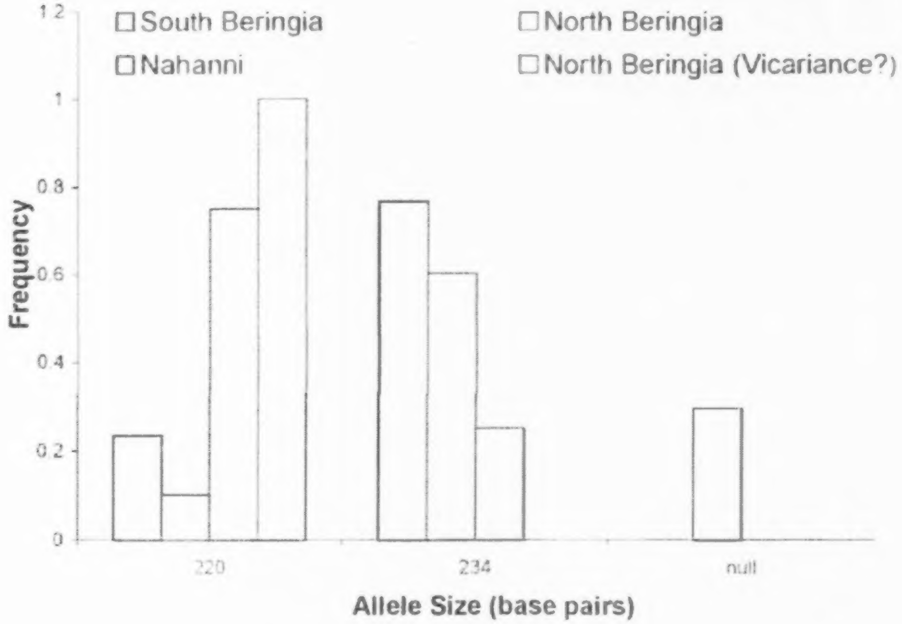
A: Alleles frequencies at the Tar1locus among glacial refugial groups.



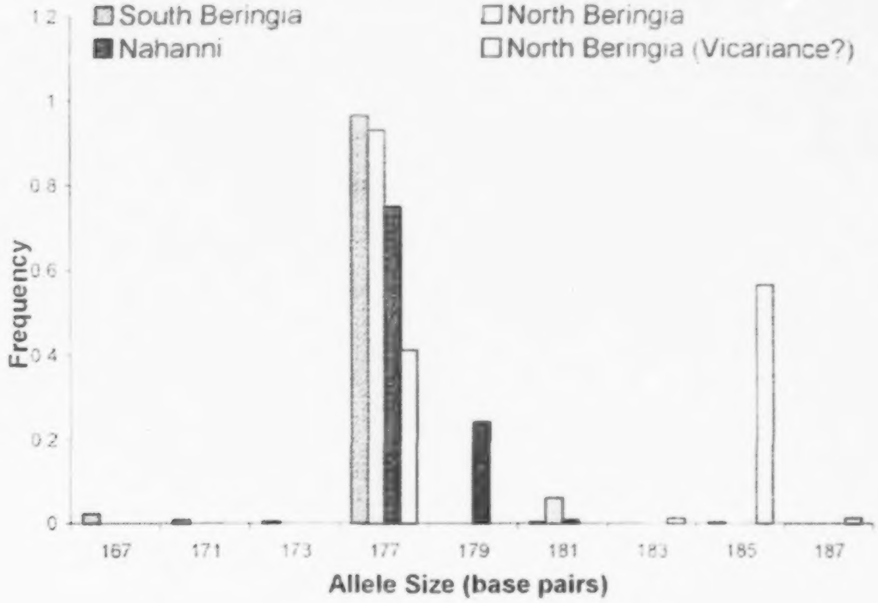
B: Allele frequencies at the *Tar8* locus among glacial refugial groups.



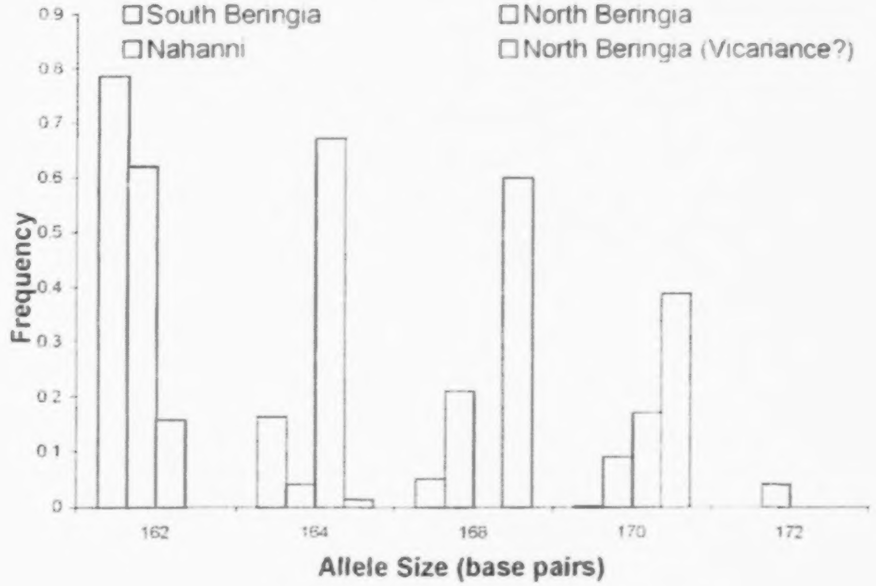
C: Allele frequencies at the Ots 100 locus among glacial refugial groups



D: Allele frequencies at the *One8* locus among glacial refugial groups.



E: Allele frequencies at the *BFR004* locus among glacial refugial groups.



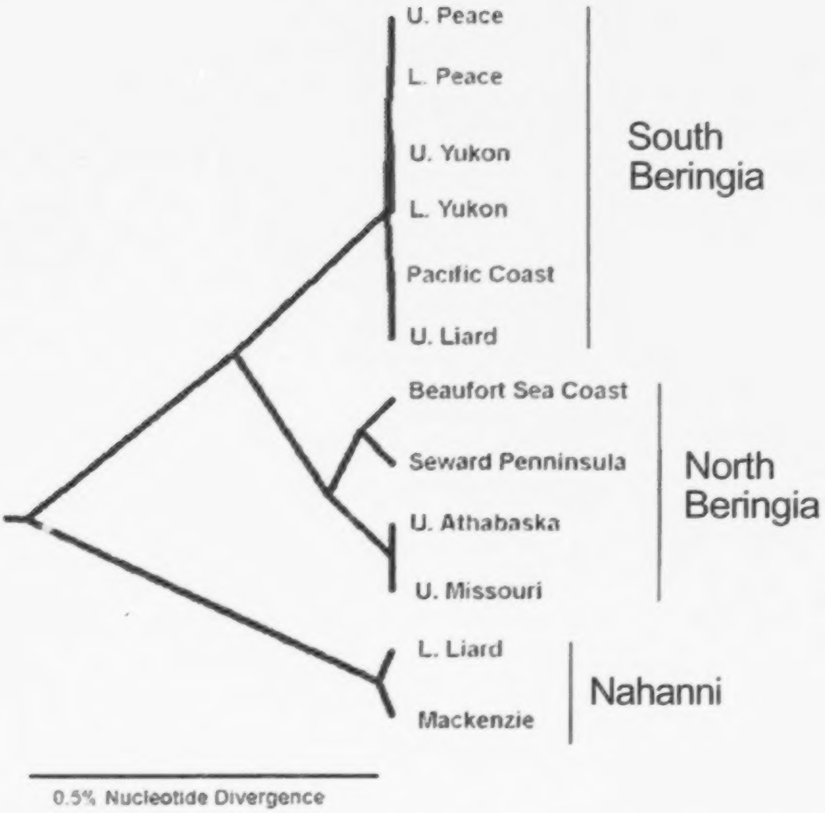

U. Peace
L. Peace
U. Yukon
L. Yukon
Pacific Coast
U. Liard
South Beringia
Beaufort Sea Coast
Seward Penninsula
U. Athabaska
U. Missouri
North Beringia
L. Liard
Mackenzie
Nahanni
0.5% Nucleotide Divergence

Figure 12: UPGMA cladogram of mtDNA nucleotide divergence estimates among regions. Population groups were suggested from clustering of mtDNA divergences. The populations that were analysed with diagnostic enzymes (see text) were included by appointing the most common haplotype from the respective mtDNA lineage (Group A, B or C see Figure 3) for each individual. Nucleotide divergence is the total branch lengths between the tips of the trees; e.g. 2% divergence between lower Liard and upper Liard populations.

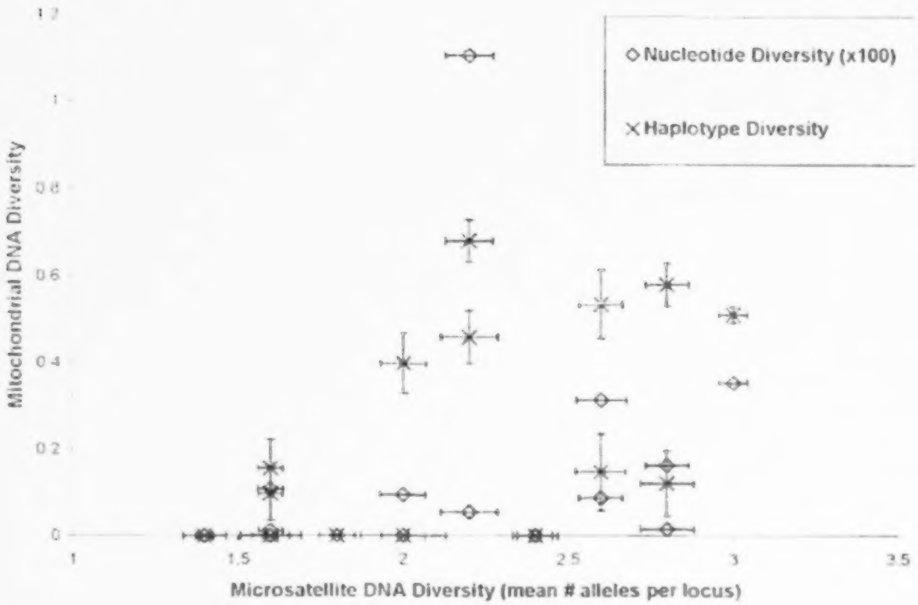


Figure 13: Relationship between microsatellite diversity (mean number of alleles per locus) and two measures of mitochondrial DNA diversity within populations. Standard error bars are shown for mean number of alleles and haplotypes diversity. Only populations where both types of markers were used were included.

Chapter 3: Local population structure of Arctic grayling in the Peace River, British Columbia.

Introduction

Loss of genetic diversity is thought to reduce evolutionary potential and increase the risk of population extinction (Dhondt 1996; Frankham 1995; Hoelzel 2000). There is, however, limited empirical evidence that associates low genetic diversity with extinction and molecular evidence has revealed that genetically depauperate populations are more common in nature than previously thought (Caughley 1994). Reasons for this may stem from the way genetic diversity is most commonly measured; i.e., at neutral DNA loci that play no role in survival. In fact, there is evidence that variation in quantitative genetic traits can increase after severe bottlenecks in both laboratory populations (Carson 1990; Whitlock and Fowler 1999) and in natural populations of postglacial origin that exhibit low genetic diversity measured with neutral molecular genetic markers (Armbruster et al. 1998). Consequently, distinct locally adapted populations may evolve within geographic regions despite the evidence that bottlenecks can cause monomorphism at neutral loci.

Postglacial populations of Arctic grayling in the Peace River were predominantly founded by a distinct lineage that survived Wisconsinan glaciation (and perhaps Illinoian) in the Yukon River Valley in Beringia (see Chapter 2). Postglacial dispersal most likely involved headwater transfer from the upper Liard River, and low genetic diversity throughout the Peace River in British Columbia probably stems from founder events associated with such headwater exchanges. Mitochondrial DNA evidence (Chapter 2) indicated a divergent haplotype below the migration barrier at Peace Canyon (which separates the upper and lower Peace River) that may have entered the lower Peace River from a glacial refuge in the upper Missouri River. Due to late westward recession of the Cordilleran ice sheet and early formation of glacial lakes at the base of the receding Laurentide ice sheet, it is likely that upper Missouri grayling were able to colonize the Peace River earlier than grayling from the Beringian lineage (Lindsey and McPhail 1986). Furthermore, these Missouri origin grayling may once have been present in the upper Peace River because early Holocene glacial lakes flooded the barrier at Peace Canyon (Lindsey and McPhail 1986). At least two species of freshwater fish originating from the upper Missouri refuge, the white sucker *(Catostomus commersoni)* and the brassy minnow *(Hybognathus hankinsoni,* McPhail and Lindsey 1970, McPhail and Carveth 1992), gained access into the upper Peace River and dispersed further west and south via glacial lakes located at present day Prince George.

Arctic grayling are absent from both the Fraser River and Skeena River watersheds and extensive analysis of mtDNA in the upper Peace (100 fish) found no evidence that Arctic grayling from the Missouri River ever colonized the upper Peace River (Chapter 2). Possibly, Arctic grayling bearing mtDNA group B entered the Peace River after the glacial lakes had drained away and never dispersed upstream of the Peace Canyon. Alternatively, Arctic grayling from the Missouri colonized the upper Peace River, but further dispersal across the divide into the Fraser River was inhibited by competition with other freshwater fish species or unsuitable ecological conditions. Catastrophic events (perhaps draining of Glacial Lake Peace) may have adversely affected the

Peace River Arctic grayling and, in combination with stochastic lineage sorting (Avise 1994), drove the Missouri mtDNA extinct in the upper Peace River. Consequently the only remaining mtDNA evidence that upper Missouri River grayling were present in the Mackenzie drainage is in the lower Peace River. A more detailed analysis of population structure with microsatellites in Peace River Arctic grayling might reveal historical influence from multiple lineages upstream of the dispersal barrier (Peace Canyon). A larger effective population size of nuclear (microsatellite) loci relative to mtDNA makes them less sensitive to loss due to drift (Hansen and Loeschcke 1996). Consequently, microsatellite alleles that are unique to Missouri River grayling (or other lineages) might still be present in some populations within the upper Peace River.

Patterns of genetic divergence among populations within the upper Peace River might reveal homing to natal spawning sites. Strong homing behaviour is expected to decrease the amount of genetic exchange among populations, which in turn increases the genetic divergence among populations (Slatkin 1993). The potamodromous (migrations within flowing freshwaters; Northcote 1997) life history of Arctic grayling requires a wide geographic range that includes specific habitats for each life history stage across different seasons. As a result, it is possible that neighbouring populations share the same habitats at some life history stage, which might increase the rate of gene flow between them. By the same logic, populations that do not share habitats should exchange fewer migrants and show greater genetic divergence assayed with molecular markers. Consequently, isolation by distance (Slatkin 1993) should be apparent, but streams that can provide habitat for all life history stages are likely to have greater genetic divergence from each other. Alternatively, if gene flow between upper Peace River tributaries is restricted (e.g. by homing behaviour or geographic barriers), the influence of genetic drift on the population structure would be high relative to migration, which can result in a non-significant correlation between pairwise geographic and genetic distances (e.g. Wenburg et al. 1998; Water et al. 2000). Furthermore, because both strong homing behaviour and geographic barriers restrict gene flow, the amount of genetic divergence between Peace River populations is predicted to be similar regardless of the presence of barriers if Arctic grayling home perfectly to their natal stream to spawn. These predictions assume that genetic divergence among populations has evolved through genetic drift and that all populations diverged from the same common ancestor (glacial refugial group).

The purpose of this chapter is to investigate population structure among Arctic grayling populations in the Peace River to gain insight into the effects of geographic barriers, geographic distance, and homing behaviour on gene flow. I examined the population structure with two independent genetic marker systems, mitochondrial DNA and microsatellite loci. The power of such an approach stems not only from enabling comparisons between two independent gene trees within the same set of populations, but these markers have different sensitivities to historical evolutionary and demographic forces. MtDNA is haploid and maternally inherited so haplotype diversity is easily lost in isolated populations through random genetic drift, bottlenecks and founder events. Consequently, mtDNA markers are especially useful for measuring genetic divergence among populations that have been isolated from each other for short periods (Avise 1994). Historical demographic events (e.g. founder events during postglacial range expansion) can severely deplete mtDNA diversity in regions (watersheds), which compromises the precision for estimating local population structure. A high mutation rate results in exceptionally high genetic diversity at microsatellite loci ($\sim 10^{-3}$ mutations per locus per generation) that is maintained in the face of genetic drift and bottlenecks due to its diploid and bi-parental inheritance (a larger effective population size, Jarne and Lagoda 1996). Consequently, microsatellite loci are a source of genetic variation for examining population structure among populations that have lost mtDNA haplotype diversity through bottlenecks and/or founder events during postglacial range expansion (Brunneretal. 1998).

Site Description

The upper Peace River is located at the southern limit of the range of Arctic grayling in British Columbia and is defined downstream by an impassable barrier at the Peace Canyon, below which the lower Peace River is located (Figure 14). Arctic grayling populations have been isolated in this watershed since they colonized the region postglacially from Beringia about 10,000 years ago. Low genetic diversity within and among populations throughout the watershed (see chapter 2) possibly resulted from bottlenecks and/or founder events during colonization.

Recent industrial development and rapid human population growth have probably affected the Arctic grayling environment in the upper Peace River, which may have disrupted historical population structure. The W.A.C. Bennett Dam was built in the Peace Canyon in 1968 and created the largest body of freshwater in British Columbia (Williston Reservoir) in the upper Peace watershed. For about a decade afterwards creel surveys and fisheries inventory studies found Arctic grayling were abundant in most of the tributary streams and large numbers congregated at the stream mouths throughout Williston Reservoir (Bruce and Starr 1985; Barrett and Halsey 1985). Soon after there was a dramatic decline in abundance, and by 1988 Arctic grayling were almost extinct in all but the larger streams (Blackman 1992). The combined effects of overfishing and habitat loss due to reservoir formation are the most likely causes of this decline (Northcote 1993). The apparent abundance of Arctic grayling soon after the dam was constructed was probably made up largely of adults and juveniles that were displaced from fluvial habitat destroyed as the reservoir filled. Such displaced grayling might have strayed to populations that were less severely impacted by creation of the reservoir. Consequently, there may have been a period of increased genetic exchange among formally genetically distinct populations.

Materials and Methods

A total of 138 and 323 Arctic grayling tissue samples were collected for mtDNA and microsatellite DNA analysis, respectively, from six tributaries from the upper Peace River (Williston Reservoir) and three tributaries of the lower Peace River in British Columbia (Table 9, 10, Figure 14). Most of the samples consisted of adipose fin clips from foraging adults collected during summer, approximately two months after spawning time (May or June). The remaining samples were fry, collected from the Table and Anzac rivers. The geographic proximity of these streams to each and radio telemetry (Blackman 1998) suggests that adult foraging and spawning site were sometimes located in different streams. Fry samples were collected from throughout the lower 20 kilometers of each river during June, July and August in 1998 to avoid collecting siblings.

Mitochondrial DNA

See Chapter 2 Materials and Methods

Microsatellites

See Chapter 2 Materials and Methods

Data Analysis

Mitochondrial DNA haplotype diversity and nucleotide diversity were calculated using the Restriction Enzyme Analysis Program *(REAP,* McElroy et al. 1992; see chapter 2 methods).

Microsatellite allele frequencies were examined for Hardy-Weinberg equilibrium within populations and for departures from linkage disequilibrium between loci using *Genepop* (Version 3.1d, Raymond and Rousset 1995). Measures of F_{st} among populations within regions and pairwise genetic differences between populations (θ, Weir and Cockerham 1984) were determined using *Genepop* and their significance from permutation procedures in *FSTAT* (version 2.8 for F_{st} within regions, Goudet 1999) and *Arlequin* (for pairwise F_{st}, Schneider et al. 1997). Pairwise differences among populations at each locus were also determined from permutation procedures in *Genepop.* The significance levels for all multiple comparisons were adjusted using the sequential Bonferonni (Rice 1989) with an initial significance level of 0.05. Analysis of molecular variance (AMOVA) between regions and within and among populations were determined using *Arlequin.* Correlation analysis between pairwise geographic and genetic (θ) distances was carried out using *Genepop.* Geographic distances were measured between sample locations (populations) along streams from a digitized map of the Peace River watershed. Mantel's test (Mantel 1967) was used to determine whether a non-random association existed between the geographic and genetic distances among the populations. Such a test is a randomization procedure that calcualtes the probability that two distance matrices are more similar than expected by chance (Sokal 1979).

Cluster analysis was carried out using Cavalli-Sforza chord distance estimates (Cavalli-Sforza and Edwards 1967) by importing a formatted continuous characters data file of allele frequency into GENDIST and building cladograms using NEIGHBOR, CONSENSE and DRAWGRAM in *Phylip* (Falsenstein 1998). The genetic distance estimates were also accompanied by 1000 bootstrap replicates generated from SEQBOOT program, and a genetic distance matrix was built for each replicate. C-S Chord distance was chosen due to its sensitivity to genetic drift relative to other distance measures. Evidence for historical bottlenecks and founder events and recent (postglacial) colonization of the Peace River watershed strongly suggests random genetic drift has been primarily responsible for levels of divergence among Arctic grayling populations (Chapter 2). The UPGMA was chosen because the results from Chapter 2 found this tree building method gave a topology with strong bootstrap support that allied closely to expectations drawn from zoogeography and the mtDNA results (see Figure 10 Chapter 2). The random nature of divergence through drift and the likelihood for variable population sizes, however, suggests the N-J method might outperform UPGMA on local population structure. Consequently, both methods were used to compare clustering among Peace River populations.

Results

Mitochondrial DNA Diversity

All but three Arctic grayling (total N = 138) in the Peace River contained the same mtDNA haplotype (haplotype 1) that was also common in Arctic grayling that survived glaciation in the Yukon River valley in south Beringia (see Table 5 Chapter 2). A unique haplotype was found in one individual from the Mesilinka River (haplotype 2, group A) and two individuals in the Burnt River contained haplotype 7 (group B). The southern distribution of haplotype 7 in Montana and Saskatchewan suggests that it originated from an upper Missouri River glacial refuge (see Chapter 2 and Redenbach and Taylor 1999). Low diversity of mtDNA in Arctic grayling throughout the Peace River (Table 9) made estimation of genetic divergence among populations impossible.

Microsatellite Diversity and the distribution of alleles

Once corrections for null alleles were made for *Tar* 1 there were no significant deviations from Hardy-Weinberg equilibrium in the Peace River populations (Table 6 and discussed in Chapter 2) and all microsatellite loci were in linkage-equilibrium. All Peace River populations were fixed, however, for allele 196 at the Tar 8 locus and for allele 177 at the *One 8* locus. Unique alleles among Peace River populations were only found in the Beatton River, which had allele 79 at the *Tar* 1 locus (also found in Saskatchewan populations, Sagavonirktok River (Beaufort Sea coast), Great Slave Lake, Yukon River (Chena River and Teslin River), and Becharof Lake and Copper River in western Alaska, see Chapter 2) and allele 170 at the *BFRO 4* locus (also found in Saskatchewan populations, lower Liard and Sagavonirktok rivers, Chapter 2). Most of the diversity in the Peace River (see Table 10) was due to allelic variation at *Tar* 1 (most common allele was 81 bp) and *Ots* 100 (the most common allele was 234 bp). Halfway River harboured the only population that was fixed for an allele at *Tar* 1 (81 bp). At the *Ots* 100 locus the Finlay River population was fixed for allele 234 and the Beatton River population was fixed for allele 220. The null allele at *Tar* 1 was most common in the Nation River population, but was also found in other Peace River populations (Anzac (adults), Mesilinka, Ingenika and Beatton rivers) as well as outside the Peace River (Saskatchewan, Great Slave Lake, and along the west coast of Alaska, Chapter 2). At the *BFRO 4* locus the Table River adult, Table River fry, Anzac River adult and Nation River populations were fixed for an allele (162 bp) that was found throughout all sample sites except the Saskatchewan populations (Chapter 2). Only one other allele at this locus was found in the upper Peace River populations (164 bp, in the Finlay River, Ingenika River, Mesilinka River and Anzac River fry) and this allele was also in the lower Peace River (Halfway River). The *BFRO 4* 164 allele was found in all populations, but with greatest frequency in the lower Liard River and Great Slave Lake (Nahanni) Arctic grayling (Figure 11, Chapter 2). Two other alleles at this locus were found in the lower Peace River but not upstream of Peace Canyon; the 168 allele was found in the Burnt River, the Halfway River and the Beatton River populations, and the 170 allele was found in the Beatton River (mentioned above). Both of these alleles were most common in the Saskatchewan populations, but the 170 allele was found in all

Arctic grayling lineages while 168bp allele was not found in the Nahanni grayling (Figure 11, Chapter 2).

Genetic divergence among Peace River populations

Estimates of genetic divergence among Peace River populations were determined from only three microsatellite loci because two (*Tar* 8 and One 8) were monomorphic in the watershed (Table 10).

Cluster analysis of genetic distance (C-S Chord Distance) estimates included populations from Great Slave Lake (Nahanni lineage) and Saskatchewan (Missouri lineage) to investigate historical genetic exchange with these populations (Figure 15). Among all Peace River populations, Arctic grayling from the Beatton River had the closest affinity with Saskatchewan (Wollaton Lake) and Great Slave Lake populations that was supported statistically (62% and 81% bootstrap support from UPGMA and N-J cladograms respectively, Figure 15). The Nation River population was also divergent from other Peace River populations and its clustering with divergent lineages (Nahanni and Missouri) was supported statistically with N-J clustering (63%) but not with UPGMA (Figure 15). There was also modest bootstrap support from the UPGMA cladogram (56%, Figure 15 A) for clustering of the remaining lower Peace populations (Burnt River and Halfway River) together with the four divergent populations (Wollaston Lake, Great Slave Lake, Nation River and Beatton River) and separate from the remaining upper Peace River populations. Both N-J (54% bootstrap support) and UPGMA (58% bootstrap support) clustered two lower Peace populations together (Burnt River and Halfway River, Figure 15). UPGMA clustering among the upper Peace River populations found the closest genetic affinity between the Mesilinka River and Anzac River adult populations (58% bootstrap support, Figure 15 A).

Analysis of molecular variance (Excoffier et al. 1992) revealed that 24.5% of the microsatellite variation occurred among all Peace River populations and 75.5% was within populations. Variation among regions was greatest when the populations were partitioned into three regions (Nation River, upper Peace River and lower Peace River) that were separated by geographic barriers (Table 11). Genetic variation among regions was lower when the Nation River population was grouped together with either upper Peace or lower Peace River populations (Table 11). F_{st} (θ, Weir and Cockerham 1984) among lower Peace River populations (located below Peace Canyon; 0.384) was greater (but not significantly, 99% bootstrap confidence intervals) than the F_{st} among all Peace River populations (0.257). F_{st} among upper Peace River populations (0.140) was significantly smaller (99% bootstrap C.I.) than the F_{st} among lower Peace and Peace rivers. When the isolated Nation River population was excluded, the F_{st} among upper Peace River populations decreased to 0.060. All F_{st} estimates were statistically significantly greater than zero ($p<0.0001$, 10,000 randomizations).

Pairwise F_{st} (θ, Weir and Cockerham 1984) estimates among Peace River populations found statistical support for distinguishing all three lower Peace River populations from each other and from all upper Peace River populations (Bonferroni adjusted $p < 0.003$, 10,000 randomizations; Table12). Furthermore, the Nation River was significantly different from all other Peace River populations ($p < 0.003$, Table 12). Among the upper Peace populations (not including Nation River), statistically significant differences were found between tributaries located at the northern (Finlay Reach) and southern (Parsnip Reach) ends of Williston Reservoir (i.e. Table and Anzac rivers versus Ingenika and Finlay rivers: Figure 14, Table 12). The Anzac River adult population, however, was not significantly different from any upper Peace River population except the Nation River (Table 12).

Isolation by distance was significant among all Peace River Arctic grayling populations (Figure 16a, r=0.628, p=0.0016 Mantel's test) and was more pronounced among those upper Peace River populations that were not separated by geographic barriers (i.e. excluding the isolated Nation River and lower Peace River populations; Figure 16b, r= 0.666, p=0.003). Geographic distances among upper Peace River populations including the Nation River, however, explained the smallest proportion of the variance in F_{st} and isolation by distance among the populations was not significant (Figure 16 c, r=0.275, p=0.067).

Discussion

Nuclear DNA insights into postglacial dispersal into Peace River

The most obvious result gathered from examining population structure of Arctic grayling in the Peace River was the effect of geographic barriers on restricting gene flow. Both lower Peace River and Nation River populations had allele frequencies that separated these populations from the upper Peace River and clustered them with Nahanni and Missouri grayling in the consensus tree (Figure 15). Furthermore, both partitioning of genetic variance (Table 11) and F_{st} estimates among population groups (Figure 17) suggest that Arctic grayling from the Nation River and lower Peace River are distinct from Arctic grayling in the upper Peace River as well as from each other.

The Peace River was predominantly founded postglacially by a Beringian lineage via headwater transfer into the upper Peace River, and mtDNA analysis (Chapter 2) suggests that the lower Peace River was founded by Arctic grayling dispersing downstream through the Peace Canyon. Judging from unique alleles *(BFRO 4* 168 allele) and divergent allele frequencies (as well as mtDNA haplotypes) found below the Peace Canyon, genetic divergence among Arctic grayling on either side of this barrier was also driven by restricted influence from Missouri refuge grayling into the upper Peace River. Most notable among the lower Peace populations was divergence of the Beatton River grayling. Here, distinct alleles *(BFRO* 4 168 and 170 alleles as well as *Tar* 1 79 and null alleles) suggest historical gene flow with both Missouri and Nahanni refugial groups of Arctic grayling (Figure 11 Chapter 2). Low elevation areas at the watershed divide between the headwaters of Beatton River and the Fort Nelson River (lower Liard River) may periodically permit passage for freshwater fish (e.g. Nahanni Arctic grayling into the Beatton River) during periods of high water. Furthermore, the low gradient and predominantly turbid conditions in Beatton River are more similar to the lower Liard River than other Peace River tributaries, suggesting an ecological barrier may limit gene flow with the lower Peace River. This influence from distinct lineages raises the possibility for local adaptations in the lower Peace River populations (especially in Beatton River) promoted by immigration of alleles from novel areas.

Although there were no unique alleles in the Nation River population, allele frequency differences made them distinct from other populations in the Peace River watershed in British Columbia. This distinction was in part due to a high frequency of the null allele at the *Tar* 1 locus that was also frequent among Arctic grayling carrying mtDNA group B, most notably those from Saskatchewan drainages that were colonized from a Missouri River refuge (Figure 11 Chapter 2). Although this suggests common ancestry with the grayling in Saskatchewan, the interpretation must be used with caution because null alleles might be paraphyletic (as all microsatellite alleles could be) but they also might represent more than one allele as they were identified by the absence of PCR product. This does not diminish the distinction of Nation River grayling from other Peace River populations because there were also allele frequency differences at *Ots* 100 (the only other variable locus in the Nation River). Gene flow upstream into the Nation River is clearly restricted, but gene flow downstream from the Nation River is possible judging from low frequency of null alleles in other upper Peace River populations and the fact that Arctic graying have been found below the barriers in the Nation River canyon (Langston and Blackman 1993).

Unique alleles in the upper Peace River populations *(BFRO 4* 164 allele and possibly Ots 100 220 allele) suggest historical influence from the Nahanni grayling. These alleles were found in high frequency in all lower Liard River populations as well as in the upper Liard River, although both were rare in Alaskan populations (Figure 11 Chapter 2). Although there was no mtDNA evidence that Nahanni grayling expanded their range into the upper Liard River watershed, it is possible that Arctic grayling dispersed past the barrier at the Liard River Canyon (Hell Gate) early in the Holocene as proposed for other Nahanni origin species of freshwater fish (Lindsey and McPhail 1986; Foote et al. 1992, Wilson and Hebert 1998). Consequently, the Nahanni influence in the upper Peace River most likely stems from Arctic grayling colonizing from the upper Liard River. A Nahanni influence in the upper Peace River is not surprising as lake whitefish from the same glacial refuge also colonized the watershed (Foote et. al 1992). However, lake whitefish were able to colonize further into the Fraser River, suggesting they were in the upper Peace River before Arctic grayling. Lake whitefish may have gained access to the upper Peace River from downstream via Glacial Lake Peace and dispersed further into Fraser River via Glacial Lake Prince George (Lindsey and McPhail 1986; Foote et al. 1992). The different dispersal routes for Arctic grayling and lake whitefish from the same glacial refuge (Nahanni) most likely reflects different habitat parameters (lake versus stream dwellers, respectively) between the species and habitat conditions in different regions early in the Holocene.

Population structure of Peace River Arctic grayling

Genetic diversity at microsatellite loci was low in populations of Peace River Arctic grayling (Table 10) relative to that in populations of other salmonid fishes in North America (e.g. Angers et al. 1995; McConnell et al. 1997; Patton et al. 1997; Bernatchez et al. 1998; Wenburg et al. 1998; Bernatchez et al. 1999; McLean 1999; Taylor et al. 2000; Taylor and McPhail 2000) and Europe (Brunner et al. 1998; Gross et al. 2001), which had greater than a mean of three alleles per locus per population (compare with Table 10). Although genetic diversity in Peace River populations was greater at microsatellite loci (Table 10) relative to that at mtDNA (Table 9), a comparable scenario for *Salvelinus alpinus* populations in Europe found a greater increase in microsatellite diversity (Brunner et al. 1998). Low diversity at microsatellite loci in Peace River Arctic grayling might be due to low mutation rates at these independent loci. Alternatively, it may be due to demographic processes that have reduced effective population sizes more severely in Arctic grayling. The latter scenario is supported by comparing the diversity at the One 8 locus between European grayling (*T. thymallus)* and the Arctic grayling. There were 10.5 alleles per locus per population (four populations) in *T. thymallus* in the Danube and Rhine rivers in Germany (Gross et al. 2001) and only 1.6 alleles per locus per population (27 populations) of *T. arcticus* in North America (Chapter 2).

A dramatic decline of genetic diversity with distance from the Yukon River Valley (the glacial origin of Peace River Arctic grayling, Chapter 2), suggests low genetic diversity in Peace River Arctic grayling resulted from founder events and/or bottlenecks during postglacial range expansion. Nonetheless, genetic diversity at microsatellite loci was also low (≤ 3 alleles per locus per population) in Arctic grayling populations located close to their putative glacial refuge (Table 2, Chapter 2). Further, mtDNA haplotype diversity was low in North American Arctic grayling (Table 1 Chapter 2) relative to other freshwater salmonid fishes in North America (e.g. Bernatchez and Dodson 1991; Wilson et al. 1996; Wilson and Hebert 1998; but see Bernatchez and Wilson 1998). This suggests local demographic processes due to aspects of Arctic grayling life history reduce effective population sizes and, consequently, have reduced intrapopulation genetic diversity at neutral genetic markers through genetic drift. Bull trout *(Salvelinus confluentus)* have similarly low genetic diversity throughout their range in North America (Taylor et al. 1999; Taylor et al. 2001) suggesting they too have been impacted by historical bottlenecks or founder events in combination with genetic drift on small effective population sizes. Both Arctic grayling and bull trout might naturally be partitioned in their environment among many small isolated populations, making them especially vulnerable to disturbances or changes to their environment (e.g. global warming and/or habitat destruction).

Genetic divergence estimates among Peace River Arctic grayling populations also suggests low levels of genetic exchange among populations. Partitioning of microsatellite molecular variance (AMOVA) among Peace River Arctic grayling populations (Table 11) was similar to that among bull trout populations (Taylor et al. 2001), landlocked Arctic char *(Salvelinus alpinus,* Brunner et al. 1998) and kokanee populations *(Oncorhyncus nerka,* Taylor et al. 2000) where the largest proportion of variation occurred within populations. Likewise, F_{st} estimates for Arctic grayling in Peace River (Figure 17) were similar to other freshwater salmonid populations (e.g. Estoup et al. 1998; Taylor et al. 2001, Gross et al. 2001) but tended to be greater than F_{st} estimates among anadromous populations (e.g. Bernatchez et al 1998; Wenburg et al. 1998; Waters et al. 2001). F_{st} estimates among Arctic grayling populations in the upper Peace River (θ=0.060, Figure 17), however, are similar to anadromous populations of Arctic char (θ=0.059, Bernatchez et al. 1998) and cutthroat trout (O. *clarki clarki,* θ ranged from 0.023 to 0.179, Wenburg et al. 1998) suggesting that geographic barriers on the Peace River and Nation River are responsible for the larger divergence of these populations. In fact, DeWoody and Avise (1999) also suggested that greater microsatellite divergence among landlocked freshwater populations, relative to anadromous populations, was due to geographic barriers and watershed divides. Nevertheless, the significant level of genetic divergence among Arctic grayling populations in the upper Peace River suggests that they home to their natal streams to spawn, as do anadromous salmonids. The large F_{st} (0.384) estimate among lower Peace River Arctic grayling populations (Figure 17) reflects the presence of barriers separating these populations. A waterfall on the Burnt River and ecological conditions in Beatton River (discussed above) likely isolate these populations from other lower Peace River populations (e.g. Halfway River).

Significant isolation by distance among upper Peace River Arctic grayling populations contrasts with the anadromous populations of O.c *clarki* and *A. sapidissima,* which had non-significant isolation by distance (Wenburg et al. 1998; Waters et al. 2001). This tight (r=0.67) correlation between pairwise geographic and genetic distances suggests that Arctic grayling in the upper Peace River have a tendency to stray among neighbouring spawning populations, but long distance effective migration is relatively rare. Alternatively, adult Arctic grayling that were displaced from flooded habitat during the 1970's, joined other populations in unimpacted streams and this period of gene flow homogenized previously distinct populations. There are still significant pairwise F_{st} measures among upper Peace River populations, however, whether gene flow periodically increased after Williston Reservoir was formed or not. Furthermore, fry populations from Parsnip River tributaries (Table and Anzac rivers) consistently had larger pairwise F_{st} estimates between them and other upper Peace River populations than did Parsnip River adult populations (Table 12). This suggests that Arctic grayling adult populations (collected from summer foraging habitat) consisted of individuals that spawn in other tributaries. Tagging studies have shown that Arctic grayling adults return annually to the same summer foraging habitat (Tack 1980, Hughes 1998), but there is also evidence that they move to different streams during spawning time (Blackman 1998). My data support the latter, but this scenario needs to be substantiated with genetic analysis of fry samples (or spawning adults) collected from all upper Peace River populations. Such analysis will likely reveal a stronger relationship between pairwise genetic and geographic distances among Arctic grayling spawning populations in upper Peace River.

Geographic barriers have clearly restricted gene flow among Arctic grayling populations in the Peace River (Figure 16, 17), but significant divergence among upper Peace River populations reflects restricted gene flow due to ecological or life history characteristics. Further, low genetic diversity throughout North America at two independent neutral DNA marker systems (mtDNA and microsatellite loci, Chapter 2) suggests that Arctic grayling are subdivided among small isolated spawning populations. Such reproductive isolation is almost certainly due to strong homing to their natal streams to spawn. Such homing behaviour is further supported by my genetic data, and radio tagging data (Blackman 1998) suggests that adults from distinct spawning populations share the same summer foraging habitats. Homing behaviour of Arctic grayling is likely facilitated by imprinting at early life history stages (Ditman and Quinn 1996) as they spend their first summer, and probably their first winter and second summer, rearing in their natal stream (Tack 1980; Northcote 1995).

Conservation Implications of Peace River Arctic grayling

Arctic grayling are clearly sensitive to anthropogenic impacts, which have recently caused extinction of populations in Michigan and decline in Montana (Armstrong 1955, Scott and Crossman 1970, Northcote 1993), and fragmentation of populations in the

Mackenzie River drainage in northern Alberta and the Northwest Territories (O'Neil 2000; Berry 2000). By being subdivided into small isolated populations, Arctic grayling have become particularly vulnerable to disturbance caused by human development. Loss of only a few adults (e.g. through harvest by anglers) removes a large proportion from small populations that are unlikely to be replaced through migration from surrounding populations. Furthermore, human disturbance to spawning and rearing habitat reduces recruitment that likely depends on benign environmental conditions within a stochastic sub-Arctic climate. Populations that are isolated upstream of geographic barriers (e.g. Nation River, Burnt River and Beatton River, Figure 14) are particularly vulnerable to disturbance, due to more complete isolation and limited habitat abundance. Such a life history strategy, however, of semi-isolated demes, facilitates local adaptation among populations within watersheds (Lynch 1996). Although genetic diversity at neutral molecular markers is a poor (likely a conservative) indicator of quantitative-genetic diversity (Lynch 1996; Pfrender 2000), genetic divergence at these two components of the Arctic grayling genome are likely somewhat correlated. Consequently, population subdivision revealed by molecular genetic markers likely reflects the potential for divergence among populations in quantitative traits (Lynch et al. 1999; Pfrender et al. 2000).

Iteroparity (repeat spawning) and a long living adult stage suggest that Arctic grayling have adapted to periodic years of low juvenile recruitment (Stearns 1983; Haugen 2000). Furthermore, such life history strategies suggest that habitat requirements for early life history stages of Arctic grayling might be narrow, requiring a delicate balance between abiotic and biotic variables (e.g. temperature, flow, stability, and an abundant invertebrate community) to facilitate growth and development. These conditions might be distinct among Arctic grayling populations in the Peace River, given the evidence for homing to their natal streams. It is, therefore, imperative for successful management of Peace River Arctic grayling to limit harvesting of adults and identify spawning and rearing locations for each population so they can be sustained.

My data suggest that the genotypes of Arctic grayling in the Peace River have been influenced by multiple lineages that colonized from distinct glacial refugia (e.g. Nahanni, Missouri and the Yukon River valley in Beringia). Each lineage likely contained unique phenotypes that evolved in the isolated environment of their glacial refugia. Consequently, quantitative genetic variation in Peace River Arctic grayling has likely been enhanced by this unique assortment of these ancestral genotypes. This genetic variation subsequently became distributed as the species expanded their range throughout the watershed. Fitness was likely increased with a demographic balance between homing behaviour and straying between spawning populations that allowed local adaptation to the extent that inbreeding and outbreeding depression were avoided (Lynch 1996, Allendorf and Waples 1996). Such local adaptation increases overall genetic diversity through divergence among populations, and raises the probability of surviving future environmental change (e.g. global warming). Population subdivision, as revealed by my data, suggests there is local adaptation among Peace River populations living in distinct environments. Consequently, loss of any remaining populations increases the risk of extinction within the watershed. The best management strategy for preserving Arctic grayling populations in the Peace River stems from understanding local adaptation by identifying differences in habitat features and requirements among populations. Any activities that artificially increase genetic exchange or cause interbreeding between populations should be avoided as forced genetic exchange between divergent populations can erode local adaptation and may contribute to the collapse of future populations through outbreeding depression (Lynch 1996, Allendorf and Waples 1996).

Table 9: Mitochondrial DNA diversity in Peace River Arctic grayling populations.

Population	Sample Size	Haplotype Diversity (SE)	Nucleotide Diversity (x100)
Upper Peace River			
Table River	20	0.0000	0.0000
Anzac River	20	0.0000	0.0000
Nation River	20	0.0000	0.0000
Mesilinka River	20	0.0974 (0.0621)	0.0114
Ingenika River	20	0.0000	0.0000
Lower Peace River			
Bumt River	24	0.1560 (0.0666)	0.1078
Beatton River	14	0.0000	0.0000

Table 10: Genetic diversity at microsatellite loci in Peace River Arctic grayling populations. N and N_a are the sample size and number of alleles per population, respectively. H_e is expected heterozygosity and Ho is observed heterozygosity per population. There were no significant departures from Hardy Weinberg equilibrium (heterozygosity deficits or excesses; Bonferroni adjusted $p<0.05$) in any population. The Parsnip River population includes all four populations from the Table and Anzac rivers (listed below) combined as one panmictic group.

Population / Locus	One8				*Ots* 100				BFRO04				*Tar* 1				*Tar* 8			
	N	Na	He	Ho	N	Na	He	Ho	N	N_a	H_e	Ho	N	N_a	H_e	H_e	N	N_a	He	H_o
Upper Peace River																				
Parsnip River	120	1	0.000	0.000	120	2	0.414	0.567	120	2	0.008	0.008	116	3	0.514	0.483	116	1	0.000	0.000
Table River Adults	30	1	0.000	0.000	30	2	0.345	0.367	30	1	0.000	0.000	30	2	0.503	0.433	30	1	0.000	0.000
Table River Fry	30	1	0.000	0.000	30	2	0.494	0.567	30	1	0.000	0.000	26	2	0.462	0.615	26	1	0.000	0.000
Anzac River Adults	30	1	0.000	0.000	30	2	0.325	0.333	30	1	0.000	0.000	30	3	0.593	0.633	30	1	0.000	0.000
Anzac River Fry	30	1	0.000	0.000	30	2	0.452	0.467	30	2	0.033	0.033	30	2	0.488	0.400	30	1	0.000	0.000
Nation River	34	1	0.000	0.000	34	2	0.506	0.471	30	1	0.000	0.000	34	3	0.497	0.441	34	1	0.000	0.000
Mesilinka River	35	1	0.000	0.000	35	2	0.357	0.457	35	2	0.029	0.029	35	3	0.649	0.657	35	1	0.000	0.000
Ingenika River	23	1	0.000	0.000	23	2	0.085	0.087	23	2	0.230	0.261	23	3	0.661	0.696	23	1	0.000	0.000
Finlay River	29	1	0.000	0.000	29	1	0.000	0.000	28	2	0.070	0.071	29	2	0.459	0.483	29	1	0.000	0.000
Lower Peace River																				
Bumt River	34	1	0.000	0.000	34	2	0.503	0.500	33	2	0.167	0.182	34	2	0.058	0.059	34	1	0.000	0.000
Halfway River	18	1	0.000	0.000	18	2	0.200	0.222	18	3	0.539	0.667	18	1	0.000	0.000	18	1	0.000	0.000
Beatton River	14	1	0.000	0.000	14	1	0.000	0.000	13	3	0.520	0.615	14	3	0.443	0.429	14	1	0.000	0.000

Table 11: Analysis of molecular variance within and among Peace River Arctic grayling populations. Regional partitioning was determined by presence of geographic barriers (e.g. Peace River Canyon and Nation River Canyon) that isolate populations from each other within the watershed. Nation River was grouped with both upper Peace and lower Peace populations.

Regional Partitioning of Populations	Upper Peace vs Lower Peace vs Nation River			Upper Peace vs Lower Peace			Upper Peace vs the rest (Nation R. and Lower Peace populations combined)		
Variance Component	**Fstat**	**Percent Variation**	**P**	**Fstat**	**Percent Variation**	**P**	**Fstat**	**Percent Variation**	**P**
Between Regions	0.223	22.28	0.002	0.177	17.71	0.005	0.117	11.67	0.002
Among populations within regions	0.128	9.95	0.000	0.184	15.13	0.000	0.196	17.32	0.000
Within populations among regions	0.322	67.78	0.000	0.328	67.16	0.000	0.290	71.01	0.000

Table 12: Pairwise F_{st} (θ, Weir and Cockerham 1984) estimates among populations (lower diagonal) and pairwise probabilities for genotypic differentiation (i.e. $P(F_{st}=0)$, 10,000 Markov chain steps; upper diagonal). Numbers inside parentheses are standard errors and significance was determined using the Bonferroni adjustment.

All Loci

	Table R. Adults	Table R. Fry	Anzac R. Adults	Anzac R. Fry	Nation R.	Mesilinka R.	Ingenika R.	Finlay R. Fry	Burnt R.	Halfway R.
Table R. Adults		0.029 (0.0090)	0.381 (0.0190)	0.667 (0.0100)	0.000 (0.0000)	0.036 (0.0087)	0.000 (0.0000)	0.169 (0.0081)	0.000 (0.0000)	0.000 (0.0000)
Table R. Fry	0.039		0.015 (0.0075)	0.245 (0.0132)	0.000 (0.0000)	0.020 (0.0098)	0.000 (0.0000)	0.000 (0.0000)	0.000 (0.0000)	0.000 (0.0000)
Anzac R. Adults	0.000	0.055		0.470 (0.0250)	0.000 (0.0000)	0.950 (0.0013)	0.107 (0.0171)	0.042 (0.0084)	0.000 (0.0000)	0.000 (0.0000)
Anzac R. Fry	0.000	0.000	0.015		0.500 (0.0090)	0.137 (0.0144)	0.001 (0.0003)	0.000 (0.0000)	0.000 (0.0000)	0.000 (0.0000)
Nation R.	0.316*	0.273*	0.263*	0.270*		0.000 (0.0000)	0.000 (0.0000)	0.000 (0.0000)	0.000 (0.0000)	0.000 (0.0000)
Mesilinka R.	0.017	0.063	0.000	0.030	0.106*		0.037 (0.0077)	0.000 (0.0000)	0.000 (0.0000)	0.000 (0.0000)
Ingenika R.	0.074*	0.166*	0.040	0.112*	0.277*	0.032		0.045 (0.0069)	0.000 (0.0000)	0.000 (0.0000)
Finlay R.	0.064	0.181*	0.067	0.118*	0.449*	0.098*	0.065		0.000 (0.0000)	0.000 (0.0000)
Burnt R.	0.227*	0.168*	0.242*	0.147*	0.399*	0.236*	0.317*	0.323*		0.002 (0.0026)
Halfway R.	0.302*	0.291*	0.303*	0.274*	0.496*	0.298*	0.278*	0.295*	0.211*	
Beatton R.	0.568*	0.464*	0.554*	0.491*	0.465*	0.519*	0.577*	0.637*	0.446*	0.519*

*=statistically significant Fst, p<0.003

Locus: *Far1*

	Parsnip R.	Nation R.	Mesilinka R.	Ingenika R.	Finlay R. Fry	Burnt R.	Halfway R.	Beatton R.
Parsnip R.		0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.217 (0.007)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Nation R.	0.387*		0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Mesilinka R.	0.036*	0.222*		0.827 (0.003)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Ingenika R.	0.050*	0.196*	0.000		0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Finlay R.	0.0011	0.431*	0.062*	0.073*		0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Burnt R.	0.264*	0.674*	0.342*	0.413*	0.263*		0.537 (0.001)	0.000 (0.000)
Halfway R.	0.274*	0.647*	0.302*	0.385*	0.284*	0.002		0.000 (0.000)
Beatton R.	0.176*	0.346*	0.141*	0.117*	0.152*	0.266*	0.257*	

*=statistically significant Fst (p<0.013)

Locus: Ots100

	Parsnip R.	Nation R.	Mesilinka R.	Ingenika R.	Finlay R. Fry	Burnt R.	Halfway R.	Beatton R.
Parsnip R.		0.0004 (0.0002)	0.3427 (0.0055)	0.0000 (0.0000)	0.0000 (0.0000)	0.0181 (0.0013)	0.0163 (0.0014)	0.0000 (0.0000)
Nation R.	0.108*		0.0096 (0.0002)	0.0000 (0.0000)	0.5000 (0.0030)	0.5043 (0.0044)	0.0001 (0.0001)	0.0000 (0.0000)
Mesilinka R.	0.0015	0.169*		0.0029 (0.0004)	0.2009 (0.0030)	0.0057 (0.0006)	0.1359 (0.0030)	0.0000 (0.0000)
Ingenika R.	0.136*	0.405*	0.110*		0.1909 (0.0017)	0.0066 (0.0003)	0.3794 (0.0025)	0.0000 (0.0000)
Finlay R.	0.126*	0.502*	0.204*	0.011		0.0000 (0.0000)	0.0168 (0.0006)	0.0000 (0.0000)
Burnt R.	0.050	0.000	0.097	0.328*	0.425*		0.0005 (0.0001)	0.0000 (0.0000)
Halfway R.	0.064	0.292*	0.029	0.010	0.123	0.216*		0.0000 (0.0000)
Beatton R.	0.570*	0.370*	0.698*	0.945*	1.000*	0.444*	0.873*	

*=statistically significant Fst (p<0.0050)

Locus: *BFRO04*

	Parsnip R.	Nation R.	Mesilinka R.	Ingenika R.	Finlay R. Fry	Burnt R.	Halfway R.	Beatton R.
Parsnip R.		1.0000 (0.0000)	1.0000 (0.0000)	0.0001 (0.0000)	0.0937 (0.0016)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Nation R.	0.000		1.0000 (0.0000)	0.0063 (0.0004)	0.2246 (0.0019)	0.0256 (0.0009)	0.0000 (0.0000)	0.0000 (0.0000)
Mesilinka R.	0.000	0.000		0.0336 (0.0012)	0.5603 (0.0016)	0.0104 (0.0008)	0.0000 (0.0000)	0.0000 (0.0000)
Ingenika R.	0.261*	0.133	0.098		0.1211 (0.0023)	0.0005 (0.0001)	0.0000 (0.0000)	0.0000 (0.0000)
Finlay R.	0.038	0.0204	0.000	0.042		0.0079 (0.0006)	0.0000 (0.0000)	0.0000 (0.0000)
Burnt R.	0.151*	0.0730	0.056	0.05	0.035		0.0001 (0.0001)	0.0000 (0.0000)
Halfway R.	0.684*	0.429*	0.418*	0.216*	0.346*	0.224*		0.0170 (0.0014)
Beatton R.	0.625*	0.722*	0.712*	0.496*	0.642*	0.527*	0.381	

*=statistically significant Fst (p<0.0036)

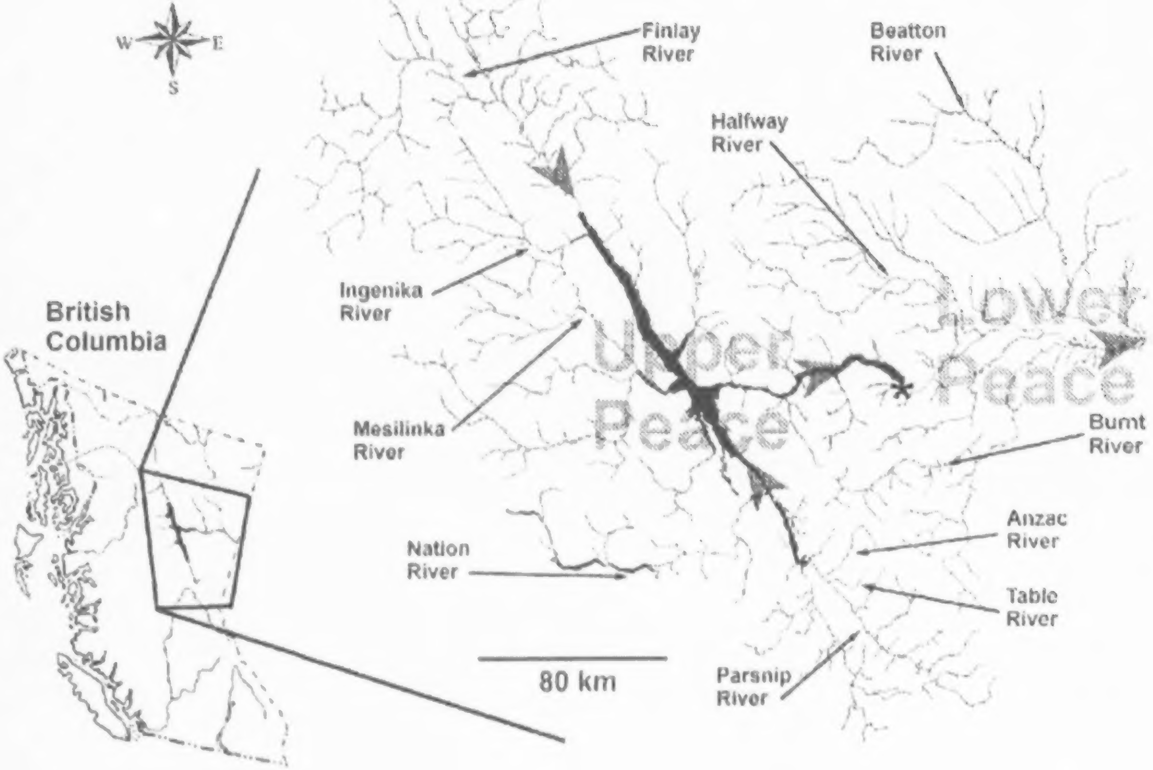

N
W
E
S
British Columbia
Finlay River
Beatton River
Halfway River
Ingenika River
Mesilinka River
Upper Peace
Lower Peace
Burnt River
Anzac River
Nation River
Table River
Parsnip River
80 km

Figure 14: Location of Arctic grayling sample sites in the Peace River watershed in British Columbia. Large arrows along streams indicate the direction of flow. The asterisk marks the location of W.A.C. Bennet Dam at Peace Canyon, which separates the upper and lower Peace rivers.

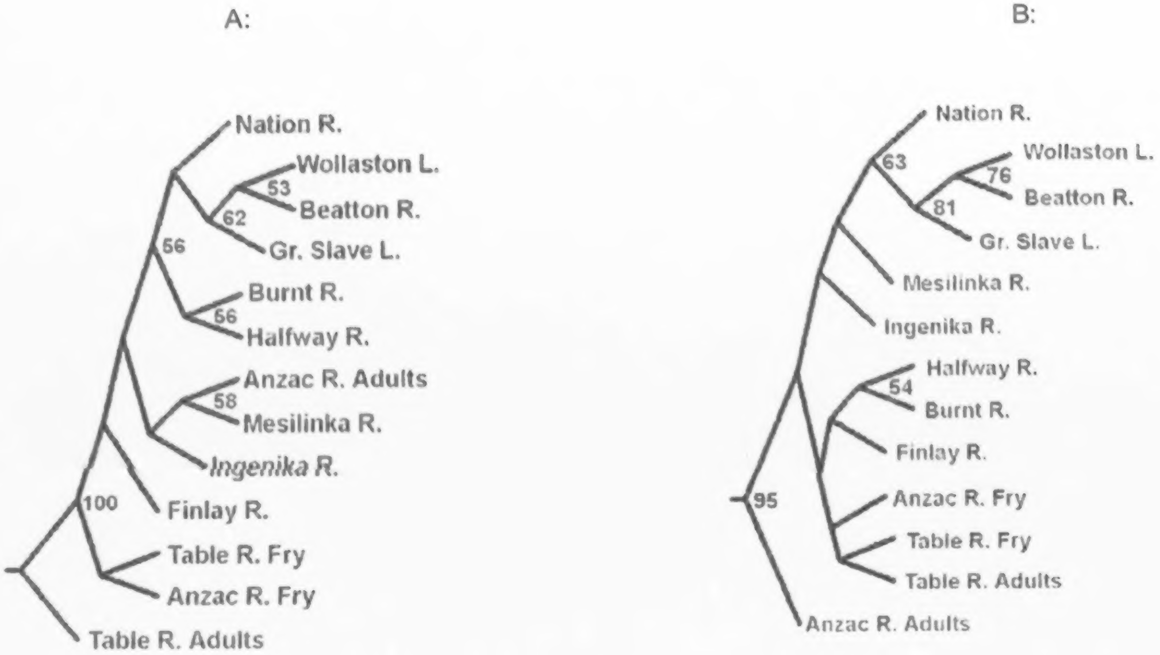

A:
Nation R.
Wollaston L.
53
Beatton R.
62
Gr. Slave L.
56
Burnt R.
56
Halfway R.
Anzac R. Adults
58
Mesilinka R.
Ingenika R.
100
Finlay R.
Table R. Fry
Anzac R. Fry
Table R. Adults
B:
Nation R.
63
Wollaston L.
76
Beatton R.
81
Gr. Slave L.
Mesilinka R.
Ingenika R.
Halfway R.
54
Burnt R.
Finlay R.
95
Anzac R. Fry
Table R. Fry
Table R. Adults
Anzac R. Adults

Figure 15: Cluster analysis of C-S Chord (Cavalli-Sforza and Edwards 1967) distance estimates calculated from microsatellite allele frequency differences among populations in the Peace River, Wollaston Lake and Great Slave Lake. A: UPGMA. B: Neighbor-Joining Method. Numbers at the branch forks are the percentage out of 1000 bootstrap replicates that the populations to the right of the branch forks occurred among the trees.

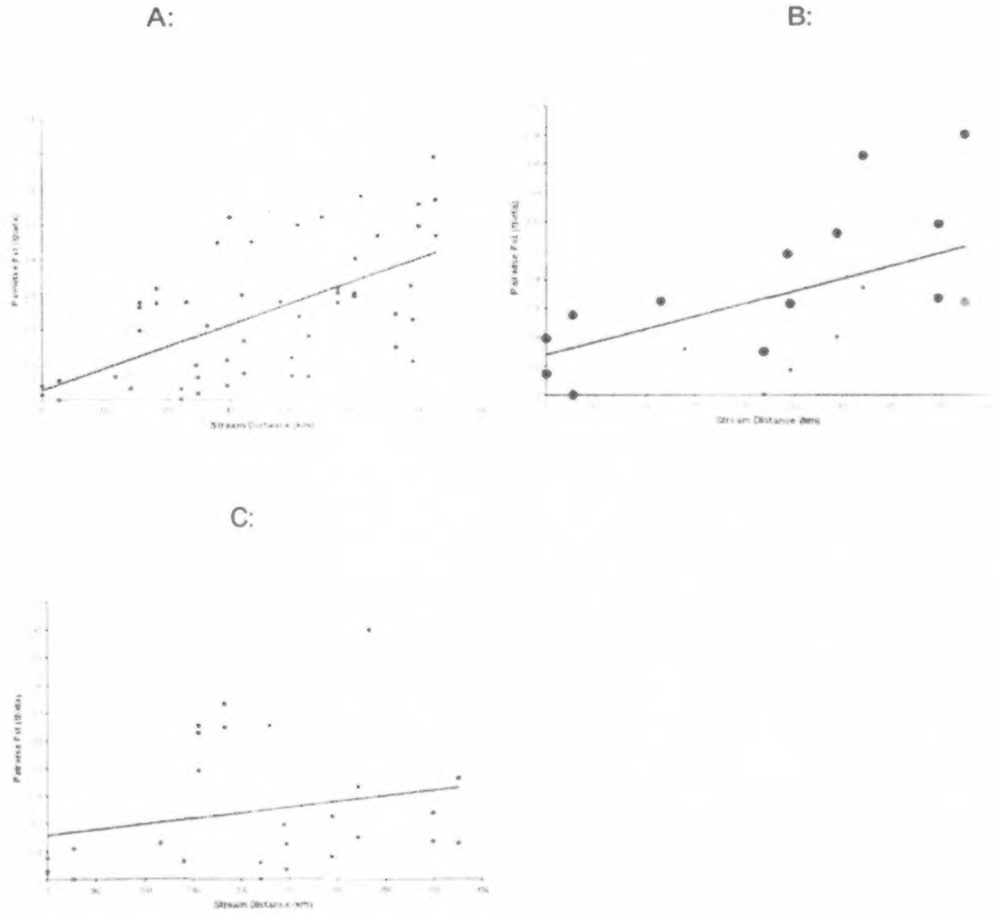


Figure 16: Pairwise stream distance (kilometers) versus pairwise F_{st} (θ, Weir and Cockerham 1984) among Peace River populations. A: Isolation by distance among all Peace River populations. B: Isolation by distance among upper Peace River populations, excluding the Nation River. Pairwise comparisons with fry populations (Parsnip River and Finlay River) are accentuated with larger data points and small points are comparisons between adult populations. C: Isolation by distance among all upper Peace populations (i.e. including the Nation River population). See text for details.

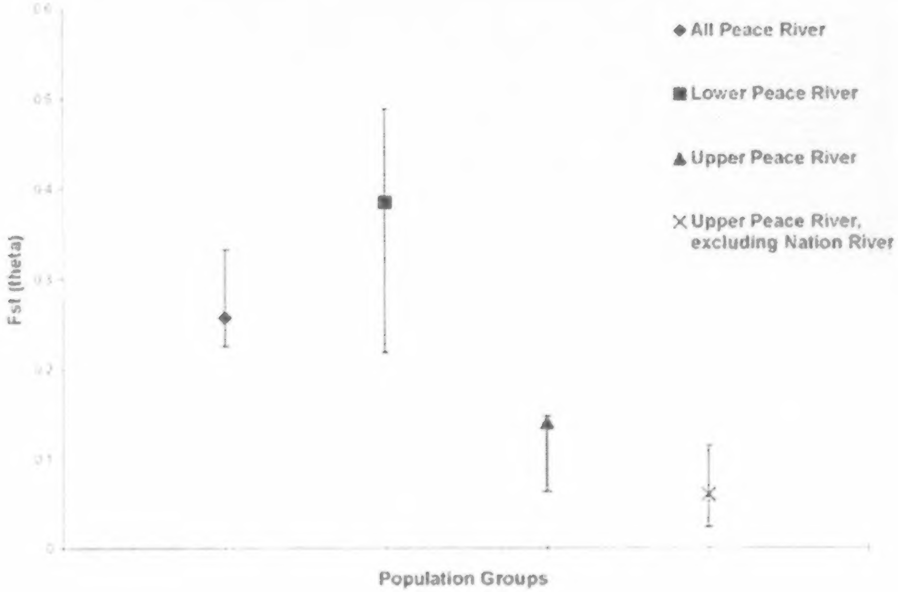


Figure 17: Comparisons between F_{st} (θ, Weir and Cockerham 1984) estimates among Peace River Arctic grayling populations grouped to show the influence of geographic barriers. The upper Peace River excluding Nation River group and lower Peace River group of populations are not separated by geographic barriers; all Peace River group included populations that are isolated by Peace Canyon and Nation River Canyon; upper Peace River group included the isolated Nation River population. Error bars are 99% bootstraped confidence intervals.

Chapter 4: General Discussion

Origin and diversification of Arctic grayling in North America

Prevailing phylogeographic and geological evidence suggests that Eurasia was impacted less severely by Pleistocene glaciation events than was North America. Furthermore, high species diversity within the genus *Thymallus* in Mongolia and Siberia suggests Eurasian ancestry for North American *T. arcticus.* Investigations aimed at determining the phylogeny of *Thymallus spp.,* however, are required to find which Eurasian species expanded their range eastward and founded *T. arcticus* in North America. The distribution and genetic divergence among North American *T. arcticus* lineages in my thesis suggests that they are polyphyletic, stemming from two episodes of colonization. If this is true then the Nahanni lineage was likely founded in North America early in the Pleistocene, and Beringian grayling founded more recently. Nahanni Arctic grayling may have been widely distributed initially in North America during early Pleistocene interglacials as suggested by the 200,000 year old fossil in Indiana. Their current more localized distribution, however, in the middle Mackenzie River (Figure 8 Chapter 2) likely reflects dramatic range contraction (perhaps multiple times) and survival in a small isolated glacial refuge. A consequence of such isolation might be a limited ability for Nahanni grayling to expand their range due to depletion of quantitative genetic diversity, which would impair their potential for adapting to novel environments.

Diversification of Beringian Arctic grayling into two widely distributed North American lineages may be associated with greater quantitative genetic diversity, relative to the Nahanni grayling (Figure 8 Chapter 2). Survival in a large ice-free region such as Beringia suggests that there was more recent contact with Siberia and greater genetic diversity has been preserved among many populations. There was no evidence in my thesis, however, that genetic diversity was lower in the Nahanni lineage (see Figure 8 Chapter 2), but diversity at quantitative genetic loci is poorly reflected by diversity at neutral molecular DNA (Lynch 1996; Lynch et al. 1999). Beringian Arctic grayling were clearly more successful at expanding their range behind the receding glaciers, and such colonization of novel environments was likely promoted by high genetic diversity at quantitative genetic loci. It is unlikely that geographic barriers restricted range expansion from the Nahanni Refuge considering the novel habitat would have opened up downstream of the refuge, and prevailing geological evidence suggests barriers restricted dispersal from Beringia (Lindsey and McPhail 1986). Further, Nahanni lineages of lake whitefish (Foote et al 1992) and lake trout (Wilson and Hebert 1996) clearly had access to drainage connection that enabled wide dispersal into separate watersheds to the south, east and west.

Although genetic exchange among distinct populations might promote outbreeding depression within established locally adapted populations (Lynch 1996; Allendorf and Waples 1996), such re-assortment of the Arctic grayling genome might facilitate colonization of novel habitats. Population subdivision within watersheds suggests that Arctic grayling home to their natal streams to spawn (Chapter 3), which facilitates adaptive radiation through local adaptation (Lynch 1996). Such population divergence requires that an initial diversity of quantitative trait loci is present within the ancestral colonizers, which then becomes partitioned among spawning populations by differential selection pressures. Patterns of molecular genetic diversity in the Peace River Arctic grayling populations suggests that the watershed was colonized by multiple lineages, most likely from a Missouri and Beringian glacial refuge (Chapter 3). Although severe bottlenecks occurred during postglacial range expansion into the Peace River, such an influence from potentially distinct phenotypes might have boosted diversity of quantitative traits and raised the long term fitness of Arctic grayling in the watershed. Consequently, current populations have potentially become adapted to a wide range of spawning habitats. My data suggest that the current genetic diversity (of neutral DNA, Chapter 3) of Arctic grayling in the Peace River is partitioned among small semi-isolated populations, and such divergence potentially reflects divergence of quantitative traits (Pfrender et al. 2000). Consequently, the evolutionary potential of Peace River Arctic grayling likely depends on the survival of many unique spawning populations in the watershed. Possibly low population subdivision within the Nahanni refuge has stifled the ability of this lineage to adapt to novel environments and expand their range postglacially.

Conservation Genetics of Arctic grayling

The distribution of mtDNA haplotypes and microsatellite alleles in my thesis revealed secondary contact among lineages within watersheds has occurred in various regions, particularly in Seward Peninsula (western Alaska) and the lower Liard River in British Columbia (see Figure 7 Chapter 2). At least two scenarios of rapid evolution are possible when potentially phenotypically distinct intraspecific groups come together postglacially: genetic exchange or introgression among groups, or further differentiation in sympatry (sympatric speciation; Schluter 1996; Taylor 1999; Bernatchez et al. 2000). For example, if evolutionary potential has been compromised somehow in one or both lineages (e.g. Nahanni lineage of Arctic grayling) then natural selection will promote interbreeding between lineages if their progeny are better able to adapt to current environmental conditions. Alternatively, the local habitat might provide enough niche space so that both lineages freely express their pre-existing phenotypic differences. Consequently, reproductive isolation and sympatric speciation between lineages could evolve.

My thesis suggests that Arctic grayling from the Nahanni refuge have limited evolutionary potential (discussed above), and that the south Beringian lineage needed to cross two barriers to reach the lower Liard River. Consequently, genetic exchange is likely between these lineages in the lower Liard River. In western Alaska, however, there is evidence for phenotypic differences (morphologically and for saltwater tolerance; Walters 1955; McCart and Pepper 1970; Tack 1980) between Arctic grayling from north and south Beringia. Furthermore, limited impacts from glaciation suggest quantitiative genetic diversity is high in Alaska. Consequently, there might be two reproductively isolated groups of Arctic grayling in Niukluk River, and the phenotypic differences between them might be exaggerated. Although information from my thesis can not differentiate between these alternative hypotheses, I have been able to identify at least two regions where these exciting phenomena may be occurring in Arctic grayling.

My thesis clearly demonstrates that evolutionary potential exists on many levels, which are not mutually exclusive. My phylogeographic analysis (Chapter 2) has identified distinct lineages that have evolved through long isolation throughout the Pleistocene. Although phenotypic characteristics were not identified, long evolution in distinct environments raises the evolutionary significance of these lineages because they are likely to possess unique and irreplaceable characteristics. On a more recent evolutionary perspective, local population subdivision (Chapter 3) suggests that many populations may also possess unique combinations of quantitative traits, which can diversify the genome and raise the likelihood for long term persistence. Such population structure might include unique characters from more than one lineage, which further diversifies the species and could increase their adaptive potential.

References

Adams, N.S., Spearman, W.J., Burger, C.V., Currens, K.P., Schreck, C.B. and L.W. Hiram. 1994. Variation in mitochondrial DNA and allozymes discriminates early and late forms of chinook salmon *(Oncorhynchus tshawytscha)* in the Kenai and Kasilof rivers, Alaska. Can. J. Fish. Aquat. Sci. 51(suppl. 1):172-181.

Angers, B., Bernatchez, L., Angers, A. and L. Degroseillers. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. J. Fish Biol. 47(Supp. A): 177-185.

Angers, B. and L. Bernatchez. 1997. Complex evolution of a salmonid microsatellite locus and its consequences in inferring allelic divergence from size information. Mol. Biol. Evol. 14(3): 230-238.

Allendorf, F.W. and R.S. Waples. 1996. Conservation and genetics of salmonid fishes. *in* J. Avise and J. Hamerick, eds. Conservation Genetics: case histories from nature. Chapman and Hall, New York.

Armbruster, P. and W.E. Bradshaw. 1998. Effects of postglacial range expansion on allozyme and quantitiative genetic variation of the pitcher-plant mosquito, *Wyeomyiasmithii*. Evol. 52(6): 1697-1704.

Armstrong R.H. 1986. A review of Arcic grayling studies in Alaska, 1952-1982. Alaska Cooperative Fisheries Research Unit, University of Alaska, Fairbanks AK 99775.

Avise, J.C., Arnold, J., Ball, J.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., and N.C. Saunders. 1987. Intraspecific phylogeny: the mitochondrial DNA bridge between populationgenetics and systematics. Ann. Rev. Syst. 18:489-522.

Avise, J.C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, London.

Avise, J.C. 1995. Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. Cons Biol. 9(3):686-690.

Banks, M.A., Blouin, M.S., Baldwin, B.A., Rashbrook, V.K., Fitzgerald, H.A., Blankenship, S.M., and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon *(Oncorhynchus tschawytscha)*. J. Hered. 90: 281-288.

Barrett, D.T. and T.G. Halsey. 1985. Fisheries resources and fisheries potential of Williston Reservoir and its tributary streams. Volume 1. B.C. Min. of Env. Fisheries Tech. Circ. 68

Beebee, T.J.C and G. Rowe. 2000. Microsatellite analysis of naterjack toad *Bufo calamita* Laurenti populations: consequences of dispersal from a Pleistocene refugium. Biol. J. Linn. Soc. 69: 367-381.

Bermingham, E. and J.C. Avise. 1986. Molecular zoogeography of freshwater fishes in the southeastern United States. Genetics 113:939-965.

Bernatchez, L. Dodson, J.J. and S. Boivin. 1989. Population bottlenecks: influence on mitochondrial DNA diversity and its effect in corigonine stock discrimination. J. Fish Biol. 35(Suppl. A):233-244.

Bernatchez, L. and J.J. Dodson. 1990. Allopatric origin of sympatric populations of lake whitefish *(Coregonus clupeaformis)* as revealed by mitochondrial DNA restriction analysis. Evol. 44(5): 1263-1271.

Bernatchez, L. and J.J. Dodson. 1991. Phylogeographic structure in mitochondrial DNA of the lake whitefish *(Coregonus clupeaformis)* and its relation to the Pleistocene glaciations.

Bernatchez, L., Colombain, F. and J.J. Dodson. 1991. Phylogenetic relationships among the subfamily Coregonidae as revealed by mtDNA restriction analysis. J. Fish. Biol. 39:283-290.

Bernatchez, L. and J.J. Dodson. 1994. Phylogenetic relationships among Palearctic and Nearctic whitefish *(Coregonus* sp.) populations as revealed by mitochondrial DNA variation. Can. J. Fish. Aquat. Sci. 51(Suppl. 1): 240-251.

Bernatchez, L. 1995. A role for molecular systematics in defining evolutionary significant units in fishes. Am. Fish Soc. Symp. 17:114-132.

Bernatchez, L. and A. Osinov. 1995. Genetic diversity of trout (genus Salmo) from its most eastern native range based on mtDNA and nuclear gene variation. Mol. Ecol. 4: 285-297.

Bernatchez, L., Vuorinen, J.A., Bodaly, R.A. and J.J. Dodson. 1996. Genetic evidence for reproductive isolation and multiple origins of sympatric trophic ecotypes of whitefish *(Coregonus)*. Evol. 50(2): 624-635.

Bernatchez, L. and C.C. Wilson. 1998. Comparative phylogeography of Nearctic and Palearctic fishes. Mol. Ecol. 7:431-452.

Bernatchez, L., Dempson, J.B. and S. Martin. 1998. Microsatellite gene diversity analysis in anadromous arctic char, *Salvelinus alpinus*, from Laborador, Canada. Can. J. Fish. Aquat. Sci. 55: 1264-1272.

Bernatchez, L., Chouinard, A. and G. Lu. 1999. Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, *Coregonus* sp., as a case study. Biol. J. Linn. Soc. 68:173-194.

Berry, D. 2000. Managing Arctic grayling in Alberta. Fish. Wildlife Management division, Alberta Env., Ed. AB. Presentation and abstract at Arctic grayling workshop. Sponsored by Peace/Williston Fish and Wildlife Compensation Program.

Blackman, B.G. 1992. Fisheries resources of Williston Reservoir twenty years after impoundment. Prov. B.C., Fish. Br. and B.C. Hydro Env. Res. Man. Rep. 35pp + appendices.

Blackman, B.G. 1998. Radio Telemetry studies of Arctic grayling migrations to overwintering, spawning and summer feeding areas in the Parsnip River watershed. Peace/Williston Fish and Wildlife Compensation Program, Report XX. 24pp. plus appendices.

Bodaly, R.A. and C.C. Lindsey. 1977. Pleistocene watershed exchanges and the fish fauna of the Peel River Basin, Yukon Territory. J. Fish. Res. Brd. Can. 34:388-395.

Bruce, P.G. and PJ. Starr. 1985. Fisheries resources and fisheries potential of Williston Reservoir and its tributary streams. Vol. II. Fisheries resources potential of Williston Lake tributaries - a preliminary overview. Prov. B.C. Fish. Tech. Circ. No. 69:100pp plus appendices.

Brunner, P.C., Douglas, M.R. and L. Bernatchez. 1998. Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arcti charr Salvelinus alpinus from central alpine lakes. Mol. Ecol. 7:209-223.

Burns, J.A. 1991. Mid-Wisconsinan vertebrates and their environment from January caves, Alberta, Canada. Quat. Res. 35:130-143.

Caughley, G. 1994. Directions in conservation biology. J. Anim. Ecol 63 (2):215-244

Carson, H.L. 1990. Increased genetic variance after a population bottleneck. TREE 5:228-230.

Cavalli-Sforza, L.L., and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evol. 32:550-570.

Cumbaa, S.L., McAllister, D.E. and R.E. Morlan. 1981. Late Pleistocene fish fossils of Coregonus, Stenodus, Thymallus, Catostomus, Lota and Cottus from Old Crow Basin, northern Yukon, Canada. Can. J. Earth Sci. 18(11): 1740-1754.

Craig, P.C. and C. Pouline. 1975. Movement and growth of Arctic grayling *(Thymallus arcticus)* and juvenile Arctic char *(Salvelinus alpinus)* in a small arctic stream, Alaska. J. of Fish. Res. Brd. Can. 32:689-697.

Crossman, E.J. and D.E. McAllister. 1986. Zoogeography of freshwater fishes of the Hudson Bay drainage, Ungava Bay and the Arctic Archipelago. *In:* The zoogeography of North American freshwater fishes. C.H. Hocutt and E.O. Wiley eds. Pp53—104. John Wiley and Sons, New York.

DeWoody, J.A. and J.C. Avise. 2000 Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. J. Fish Biol. 56:461-473.

Dias, P.C., Verheyen, G.R. and M. Raymond. 1996. Source-sink populations in Mediterranean blue tits: evidence using single locus minisatellite probes. J. Evol. Biol. 9:965-978.

Dillinger, R.E. Jr., Birt, T.P., Green, J.M., and W.S. Davidson. 1991. Postglacial dispersal of longnose suckers, *Catastomus catastomus,* in the Mackenzie and Yukon drainages. Biochem. Syst. Ecol. 19(8): 651-657.

Dittman, A.H. and T.P. Quinn. 1996. Homing in Pacific salmon: mechanisms and ecological basis. J. Exp. Biol. 199:83-91

Dyke, A.S., and V.K. Prest. 1987. Late Wisconsisnan and Holocene history of the Laurentide Ice Sheet. Geog. Phys. Quat. 41: 237-263.

Ellegren, H. 2000. Microsatellite mutations in the germline: implications for evolutionary inference. Trends Genet. 16(12):551-558

Estoupe, A., Presa, P., Kreig, F., Vaiman, F., and R. Guyomard. 1993. (CT)n and (GT)n microsatellites; a new class of genetic marker for *Salmo trutta* L. (brown trout). Hered. 71:488-496.

Estoup, A., Rouset, F., Michalakis, Y., Cornuet, J.M., Adriamanga, M. and R. Guyomard. 1998. Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout *(Salmo trutta).* Mol. Ecol. 7:339-353.

Excoffier, L., Smouse, P.E. and J.M. Quattro. 1992. Analysis of molecular variance inferred from the metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Falsenstein, J. 1993. Phylip (Phylogeny Inference Package) version 3.5c. Distributed by author. Dept, of Genetics, University of Washington, Seattle, Wash.

Ford, D. C. 1976. Evidence of multiple glaciations in South Nahanni National Park, Mackenzie Mountains, Northwest Territories. Can. J. Earth Sci. 13:1433-1445.

Foote, C.J., Clayton, J.W., Lindsey, C.C. and R.A. Bodaly. 1992. Evolution of lake whitefish *(Coregonus clupeaformis)* in North Americaduring the Pleistocene: evidence for a Nahanni glacial refuge race in the northern Cordilleran region. Can J. Fish. Aquat. Sci. 49:760-768.

Fowler, K. and M.C. Whitlock. 1999. The distribution of phenotypic variance with inbreeding. EVOL 53(4): 1143-1156

Frankham, R. 1995. Inbreeding and extinction - a threshold effect. Cons. Biol. 9 (4):792-799.

Glenn, T.C. 1995. Microsatellite manual 1995. See Curtis and Taylor *IN* MOLECULAR ECOLOGY 9(1): 116-118

Goldstein, D.B. and D.D. Pollock. 1997. Launching microsatellites: a review of mutation processes and methods of phylogenetic inference. J. Hered. 88:335-342.

Goudet. 1999. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.8). *updated from* Goudet, J. 1995. FSTAT (ver. 1.2): a computer program to calculate F-statistics. J. Hered. 86:485-486.

Green, D.M., Sharbel, T.F., Kearsley, J. and H. Keiser. 1996. Postglacial range fluctuation, genetic subdivision and speciation in the western North American spotted frog complex, Rana pretiosa. Evol. 50(1):374-390.

Gross, R., Kuhn, R, Baars, M., Schroder, W., Stein, H. andO. Rottmann. 2001. Genetic differentiation of european grayling populations across the Maine, Danube and Elbe drainages in Bavaria. J. Fish Biol. 58:264-280.

Haas G.R. and J.D. McPhail. 1991. Systematics and distributions of dolly varden *(Salvelinus malma)* and bull trout *(Salvelinus confluentus)* in North America. CJFAS 48(11): 2191-2211.

Hansen M.M. and V. Loeschcke. 1996. Temporal variation in mitochondrial DNA haplotype frequencies in a brown trout (Salmo Trutta L) population that shows stability in nuclear allele frequencies. Evol. 50(1):454-457.

Hewitt, G.M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol. J. Linn. Soc. 58: 247-276.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405: 907-913

Haugen, T.O. 2000. Growth and survival effects on maturation pattern in -populations of grayling with recent common ancestors. Oikos 90:107-11.

Hedrick, P.W. 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. Evol. 53(2):313-318.

Hoelzel, R.A. 1999. Impact of population bottleneck on genetic variation and the importance of life-history; a case study of the northern elephant seal. Biol. J. Linn. Soc. 68: 23-39.

Hop, H. 1985. Stock identification and homing of Arctic grayling *Thymallus arcticus* (Pallus) in interior Alaska. M.Sc, thesis, Univ. Alaska, Fairbanks, AK. 220p.

Hop, H. and A.j. Gharrett. 1989. Genetic relationships of Arctic grayling in the Koyukuk and Tanana rivers, Alaska. Trans. Am. Fish. Soc. 118:290-295.

Hughes, N. F. 1998. Use of whole-stream patterns of age segregation to infer the interannual movements of stream salmonids: A demonstration with arctic grayling in an interior Alaskan stream. Trans. Am. Fish. Soc. 127(6): 1067-1071.

Ibrahim, K.M., Nichols, R.A. and G.M. Hewitt. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Hered. 77: 282-291.

Jarne, P. and J.L. Lagoda. 1996. Microsatellites, from molecules to populations and back. TREE 11(10):424-429.

Koskinen, M.T., Ranta, E., Piironen, J., Veselov, A., Titov, S., Haugen, T.O., Nilsson, J., Carlstein, M. and C.R. Primmer. 2000. Genetic lineages and postglacial colonization of grayling *(Thymallus thymallus,* Salmonidae) in Europe, as revealed by mitochondrial DNA analyses. Mol. Ecol. 9:1609-1624.

Kaya, C.M. and E.D. Jeanes. 1995. Retention of adaptive rheotactic behaviour by F-1 fluvial arctic grayling. Trans. Am. Fish. Soc. 124:453-457.

Kaya, C.M. 1989. Rheotaxis of young arctic grayling from populations that spawn in inlet and outlet streams of a lake. Trans. Am. Fish. Soc. 118:474-481.

Kristiansen, H. and K. B. Doving. 1996. The migration of spawning stocks of grayling *Thymallus thymallus,* in Lake Mj sa, Norway. Env. Biol. Fish. 47:43-50.

Langston, A.R. and B.G. Blackman. 1993. Fisheries resources and enhancement potentials of selected tributaries of the Williston Reservoir. Vol. II. Peace/Williston Fish and Wildlife Compensation Program Report No. 70. 185pp plus appendicies.

Lindsey, C.C. 1981. Stocks are chameleons: plasticity in gill rakers of coregonid fishes. Can. J. Fish. Aquat. Sci. 38:1497-1506.

Lindsey, C.C. and J. D. McPhail. 1986. Zoogeography of fishes of the Yukon and Mackenzie basins. In The zoogeography of North American freshwater fishes. C. H. Hocutt and E.O. Wiley, eds., John Wiley and Sons, Inc. pp.639-674.

Lindsey, C.C., Patalas K., Bodaly R.A. and C.P. Archibald. 1981. Glaciation and the physical, chemical and biological limnology of Yukon lakes. Can. Tech. Rep. Of Can. J. Fish. Aquat. Sci. No. 966.

Lynch, J.C. and E.R. Vyse. 1979. Genetic variability and divergence in grayling, *Thymallus arcticus.* Genetics 92:263-278.

Lynch, M. 1996. A quantitative-genetic perspective on conservation issues. Pp 471-501 *in* J. Avise and J. Hamerick, eds. Conservation Genetics: case histories from nature. Chapman and Hall, New York.

Lynch, M., Pfrender, M., Spitze, K., Lehman, N., Hicks, J., Allen, D., Latta, L., Ottene, M., Bogue, F. and J. Colbourne. 1999. The quantitative and molecular genetic architecture of a subdivided species. Evol. 53(1): 100-110.

Makoedov, A.N. 1987. Interpopulational differences and distribution og grayling, *Thymallus',* study of colour variation of dorsal fin. J. Ichthyology 28:24-30.

Mantel, N. 1967. The detection of disease clustering and a generalized regression apprach. Cancer Res. 27:209-220.

Mayr, E. 1982. Process of speciation. *In* Mechanisms of speciation. C. Barigozza *Ed.* Alan R. Liss Inc., New York. Pp 1-19.

McAllister, D.E. and C.R. Harrington. 1969. Pleistocene grayling, *Thymallus,* from Yukon, Canada. Can J. Earth Sci. 6:1185-1190.

McCart, P. and V.A. Pepper. 1971. Geographic variation in the lateral line scale counts of the Arctic grayling, Thymallus arcticus. J. Fish. Res. Bd. Can. 28: 749-754.

McCart, P., Craig, P. and H. Bain. 1972. Report on fisheries investigations in the Sagavanirktok River and neighboring drainages. Rep. Alaska Pipeline Serv. Co. Bellewye, Washington.

McConnell, S.K.J, Ruzzante, D.E., O'Reilly, P.T., Hamilton L. and J.M. Wright. 1997. Microsatellite loci reveal highly significant genetic differentiation among Atlantic salmon *(Salmo salar)* stocks from the east coast of Canada. Mol. Ecol. 6:1075-1089.

McElroy, D., Moran, P., Bermingham, E., and I. Kornfield. 1992. REAP: an integrated environmentfor the manipulation and phylogenetic analysis of restriction data.. J. Hered. 83:157-158.

McLean, J.E. and E.B. Taylor. 1999. Marine population structure in an anadromous fish: life history influences patterns of mtochondrial DNA variation in the eulachon, *Thaleichthys pacificus.* Mol. Ecol. 8:S143-S158.

McPhail, J.D. and C.C. Lindsey. 1970. Freshwater fishes of northwestern Canada and Alaska. Fish. Res. Brd. Can. *Bull* 173.

McPhail, J.D. and R. Carveth. 1992. A foundation for conservation: the nature and origin of the freshwater fish fauna of British Columbia. Fish Museum, Deept. Zool. UBC.

McPhail, J.D. and E.B. Taylor. 1999. Morphological and genetic variation in nortwestern longnose suckers, *Catostomus catostomus'*. The Salish Sucker problem. Copeia 4:884-893.

Merila, J., Bjorklund, M. and A.J. Baker. 1996. Genetic structure and gradual northward decline of genetic variability in the greenfinch (Carduelis chloris). Evol. 50(6): 2548-2557.

Miller, B.B., D.F. Palmer, W.D. McCoy, A.J. Smith and M.L. Colburn. 1993. A pre-Illinoian fossil assemblage from near Connersville, Southeastern Indiana. Quat. Res. 40:254-261.

Morris, D.B., Richard, K., and J.M. Wright. 1996. Microsatellites from rainbow trout *(Oncorhynchus mykiss)* and their use for genetic study of salmonids. Can. J. Fish. Aquat. Sci. 53 ;120-126.

Moritz, C. 1994. Defining 'evolutionary significant units' for conservation. TREE 9(10):373-375.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, NY.

Nei, M. and F.Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97:145-167.

Nei, M. and F. Tajima. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. Genetics 105: 207-217.

Nei, M. and J.C. Miller. 1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. GENETICS125 (4):873-879

Nelson R.J. and T.D.Beacham. 1999. Isolation and cross species amplification of microsatellite DNA useful for study of pacific salmon. Anim. Genet. 30:228-229.

Northcote, T.G. 1993. A review of management and enhancement options for the Arctic grayling *(Thymallus arcticus)* with special reference to the Williston Reservoir watershed in British Columbia. Province of British Columbia, Ministry of Environment Lands and Parks, Fisheries Branch. Fisheries Management Report No. 101: 69pp.

————————. 1995. Comparitive biology and manangement of Arctic and European grayling (Salmonidae, *Thymallus)*. Reviews in Fish Biol, and Fish. 5:141-194.

Northcote, T.G. 1997. Potamodromy in Salmonidae- Living and moving in the fast lane. North. Amer. J. Fish. Man. 17: 1029-1045.

O'Neil, J. 2000. Distribution and status of Arctic grayling in Alberta. R.L.&L Environmental Services Ltd., Edmonton AB. Presentation and abstract at Arctic grayling workshop. Sponsored by Peace/Williston Fish and Wildlife Compensation Program.

O'Reilly, P.T., Hamilton, L, McConnell, L, and J.M. Wright. 1996. Rapid analysis of genetic variation in Atlantic salmon *(Salmo salar)* by PCR multiplexing of dinucleotide and tetranucleotide micro-satellites. Can. J. Fish. Aquat. Sci. 53:2292-2298.

Orr, M.R. and T.B. Smith. 1998. Ecology and speciation. TREE 13(12):502-506.

Orti, G., Devon, EP. and J.C. Avise. 1997. Phylogenetic assessment of length variation at a microsatellite locus. Proc. Nat. Acad. Sci. 94:10745-10749.

Park, L.K., Brainward, M.A., Dightman, D.A. and G.A. Winans. 1993. Low levels of intraspecific variation in the mitochondrial DNA of chum salmon *(Oncorhynchus keta).* Mol.Mar. Biol. Biotech. 2:362-370.

Park, L.K. and P. Moran. 1994. Developments in molecular-genetic techniques in fisheries. Rev. Fish Biol. Fish. 4(3):272-299.

Patton, J.C., Gallaway, B.J., Fechhelm, R.G. and M.A. Cronin. 1997. Genetic variation of microsatellite and mitochondrial DNA markers in broad whitefish *(Coregonus nasus)* in the Colville and Sagavanirktok rivers in northern Alaska. CJFAS 54:1548-1556.

Pfrender, M.E., Spitze, K., Hicks, J., Morgan, K., Latta, L. and M. Lynch. 2000. Lack of concordance between genetic diversity estimates at the molecular and quantitative-trait levels. Cons. Gen. 1 (3):263-269.

Paetkau, D., Waits, L.P., Clarkson, P.L., Craighead and C. Strobeck. 1997. An empirical evaluation of genetic distance statisitics using microsatellite data from bear(Ursidae) populations. Genetics 147:1943-1957.

Petit, E., Excoffier, L. and F. Mayer. 1999. No evidence of bottleneck in the postglacial recolonization of Europe by the Noctule Bat *(Nyctalus noctula).* Evol. 53(4): 1247-1258.

Pielou, E.C. 1991. After the ice age: the return of life to glaciated North America. Univ. of Chicago Press, Chicago.

Raymond, M. and F. Rousset. 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86:248-249.

Redenbach, Z. and E.B. Taylor. 1999. Zoogeographical implications of variation in mitochondrial DNA of Arctic grayling *(Thymallus arcticus).* Mol. Ecol. 8-23-35.

Reed, R.J. 1973. Analysis of some meristic and morphometric datafrom the Arctic grayling, Thymallus arcticus, in Alaska. Copeia 4: 819-822.

Reiss, R.A., Ashworth, A.C. and D.P. Schwert. 1999. Molecular genetic evidence for post-Pleistocene divergence of populations of the arctic-alpine ground beetle *Amara* alpina (Paykul) (Coloeptera: carabidae). J. Biogeog. 26:785-794.

Rempel, L.L. and D.G. Smith. 1998. Postglacial fish dispersal from the Mississippi refuge to the Mackenzie River basin. Can. J. Fish. Aquat. Sci. 55:893-899.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evol. 43:223-335.

Ryman, N., Allendorf, F.W. and and G Stahl 1979. Reproductive isolation with little genetic divergence in sympatric populations of brown trout *(Salmo trutta).* Genetics 92:247-262.

Sage, R.D. and J.O. Wolff. 1986. Pleistocene glaciations, fluctuating ranges, and low genetic variability in a large mammal(Ovis dalli). Evolution 40:1092-1095.

Schluter, D. 1996. Ecological causes of adaptive radiation. Am. Nat. 148: S40-S64.

Schneider, S., Kueffer, J. M., Roessli, D., and L. Excoffier. 1997. Arlequin version 1: an exploratory population genetics software environment. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Schoffmann, J. 2000. The grayling species (Thymallinae) of three different catchment areas of Mongolia. The Grayling Society Journal, Spring 2000 issue.

Scott, W.B. and E.J. Crossman. 1973. Freshwater fishes of Canada. Fish. Res. Brd. Can. Bull. 184.

Scribner, K.T., Gust, J., and R.L. Fields. 1996. Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic aplications. Can. J. Fish. Aquat. Sci. 53:833-841.

Skopets, M.B. 1990. Biological characterisitics of the Arctic grayling in northeastern Asia. I. The Kamchatkan grayling - *Thymallus arcticus mertensi.* Journal of Ichthyology 30: 43-58.

Skopets, M.B. 1991. Biological features of a subspecies of the Arctic grayling in northeast Asia. 2. The Alaskan grayling, *Thymallus arcticus signifer.* J. Ichthyology 31:87102.

Slatkin, M. 1993. Isolation by distance in equilibrium and non equilibrium populations. Evolution 47:264-279.

Sneath and Sokal. 1973. UPGMA

Saitou and Nei 1987.

Sokal, R. R. 1979. Testing statisitcal significanceof geographic variation patterns. Syst. Zool. 28:227-232.

Stearns, S.C. 1983. A natural experiment in life-history evolution: field data on the introduction of mosquito fish *(Gambusi affinis)* to Hawaii. Evolution 37:601-617.

Stearns, S.C. and R.D. Sage. 1980. Maladaptation in a marginal population of mosquito fish, Igambousia affinis. Evol. 34:65-75.

Stuart, K.M. and G.R. Chislett. 1979. Aspects of the life histroy of Arctic grayling in the SukunkaDrainage. Report for British Columbia Fish and Wildlife, Prince George. 75pp. Plus appendices.

Tack, S.L. 1974. Distribution, abundance and natural history of the Arctic grayling in the TannanaRiver drainage. Alaska Dept. Fish and Game, Annual Progress Report 1973-1974. Project F-9-6, 15(R-1), 35pp.

Tack, S.L. 1980. Migration and distributions of Arctic grayling, *Thymallus arcticus* (Pallus), in interior and Arctic Alaska. Alaska Dept. Fish and Game, Annual Perfomance Report 1971-1980. Project F-9-12, R-I. 32pp.

Taggart, J.B., Hynes, R.A., Prodohl, P.A. and P.A. Ferguson. 1992. A simplified protocol for routine total DNA isolation frfom salmonids. J.Fish Biol. 40(6): 963-965.

Taylor, E.B. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquacult. 98: 185-207.

Taylor, E.B. 1999. Species pairs of north temperate freshwater fishes: evolution, taxonomy and conservation. Rev. Fish Biol. And Fish. 9:299-324.

Taylor, E.B., and P. Bentzen. 1993. Evidense for multiple origins and sympatric divergence of trophic ecotypes of smelt *(Osmerus)* in northeastern North America.. Evol. 47:813-832.

Taylor, E.B., Harvey, S., Pollard, S., and J. Volpe. 1997. Postglacila genetic differentiationof reproductive ecotypes of kokanee *Oncorhynchus nerka* in Okanagan Lake, British Columbia. Mol. Ecol. 6:503-517.

Taylor, E.B., Pollard, S, and D. Louie. 1999. Mitochondrial DNA variation in bull trout *(Salvelinus confluentus)* from northwestern North America: implications for zoogeography and conservation. Mol. Ecol. 8:1155-1170.

Taylor, E.B., Kuiper, A., Troffe, P.M., Hoysak, D.J. and S. Pollard. 2000. Variation in developmental biology and microsatellite DNA in reproductive ecotypes of kokanee, *Oncorhynchus nerka:* Implications for declining populations in a large British Columbia lake. Cons. Genet.

Taylor, E.B. and J.D. McPhail. 2000. Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. Proc. Roy. Soc. Lond. Series B, 267(1460):2375-2384.

Taylor, E.B., Redenbach, Z., Costello, A.B., Pollard, S.M. and CJ. Pacas. 2001. Nested analysis of genetic diversity in northwestern North American char, Dolly Varden *(Salvelinus malma)* and bull trout *(Salvelinus confluentus)*. Can. J. Fish. Aquat. Sci. 58(2): 406-420.

Thompson, C.E., Taylor, E.B. and J.D. McPhail. 1997. Parallel evolution of lake-stream pairs of three spine sticklebacks *(Gasterosteus)* inferred from mitochondrial DNA variation. Evol. 51(6): 1955-1965.

Turgeon, Estoup, A. and L. Bernatchez. 1999. Species flock in the north American Great Lakes: molecular ecology of Lake Nipigon ciscoes (Teleostei: Coregonidae: *Coregonus)*. Evol. 53(6): 1857-1871.

Waits, L.P., Talbot, S.L., Ward, R.H. and G.F. Shields. 1998. Mitochondrial DNA phylogeography of the North American brown bear and implications for conservation. Cons. Biol. 12(2): 408-417.

Walters, V. 1955. Fishes of western Arctic America and eastern Arctic Siberia. Arner. Mus. Nat. Hist. Bull. 106(5).

Waters, J.M., Epifanio, J.M., Gunter, T. and B.L. Brown. 2000. Homing behaviour facilitates subtle genetic differentiation among river populations of *Alosa sapidissima'.* microsatellites and mtDNA. J. Fish Biol. 56:622-636.

Weir, B.S. and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evol. 38:1358-1370.

Wenburg, J.K., Bentzen, P. and CJ. Foote. 1998. Microsatellite analysis of genetic population structure in an endangered salmonid: the coastal cutthroat rtrout *(Oncorhynchus clarki clarki)*. Mol. Ecol. 7:733-749.

West, R.L., Smith, M.W., Barber, W.E., Reynolds, J.B. and H. Hop. 1992. Autumn migration and overwintering of Arctic grayling in coastal streams of the Arctic national wildlife refuge, Alaska. Tran. Am. Fish. Soc. 121: 709-715

Whitlock, M.C. and D.E. MacCauley. 1990. Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. Evol. 44:1717-1724.

Whitlock M.C. and K. Fowler. 1999. The changes in genetic and environmental variance with inbreeding in *Drosophila melanogaster*. Genetics152 (1):345-353

Wilson, C.C., Hebert, P.D.N., Reist, J.D. and J.B. Dempson. 1996. Phylogeography and postglacial dispersal of Arctic charr *Salvelinus alpinus* in North America. Mol. Ecol. 5:187-197.

Wilson, C.C. and P.D.N. Hebert. 1998. Phylogeography and postglacial dispersal of lake trout *(Salvelinus namaycush)* in North America. Can. J. Fish. Aquat. Sci. 55: 1010-1024.

Wood, C.C., and CJ. Foote. 1996. Genetic differentiation of sympatric anadromous and non-anadromous morphs of sockeye salmon *(Oncorhynchus nerka)*. Evol. 50:1265-1279.

Zink, R.M. and D.L. Dittmann. 1993. Gene flow, refugia and evolution of geographic variation in the song sparrow (Melospiza melodia). Evolution 47:717-729.